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CONTENTS OF Vol. 48, No. 1

		PAGE
1.	Field trials of the effect of aldrin on clubroot of summer cabbage. By A. G. CHANNON and W. G. KEYWORTH	1
2.	Studies of the biology of *Sclerotium cepivorum* Berk. IV. Germination of sclerotia. By J. R. COLEY-SMITH. (With Plate 1 and 2 Text-figures)	8
3.	The incidence of certain seed-borne diseases in commercial seed samples. II. Ergot, *Claviceps purpurea* (Fr.) Tul., in cereals. By GILLIAN M. MARSHALL	19
4.	The incidence of certain seed-borne diseases in commercial seed samples. III. *Septoria* leaf spot, or blight, of celery. By GILLIAN M. MARSHALL	27
5.	The incidence of certain seed-borne diseases in commercial seed samples. IV. Bunt of wheat, *Tilletia caries* (DC.) Tul. V. Earcockles of wheat, *Anguina tritici* (Stein.) Filipjev. By GILLIAN M. MARSHALL	34
6.	The determination of the reaction of barley varieties to loose smut. By M. M. S. MALIK and C. C. V. BATTS. (With 3 Text-figures)	39
7.	The coliform bacteria associated with potato black-leg and other soft rots. I. Their pathogenicity in relation to temperature. By D. C. GRAHAM and W. J. DOWSON. (With Plate 2)	51
8.	Coppicing experiments on the spread and control of cacao swollen-shoot disease in Nigeria. By J. M. THRESH and R. M. LISTER. (With 2 Text-figures)	65
9.	The use of a multiple-transfer method in plant virus transmission studies—some statistical points arising in the analysis of results. By A. J. GIBBS and J. C. GOWER. (With 2 Text-figures)	75
10.	The toxicity of zinc sulphate to rainbow trout. By R. LLOYD. (With 5 Text-figures)	84
11.	The effect on wood-pigeon breeding of systematic nest destruction. By R. K. MURTON. (With 1 Text-figure)	95
12.	Movement of eelworms. VI. The influences of soil type, moisture gradients and host plant roots on the migration of the potato-root eelworm *Heterodera rostochiensis* Wollenweber. By H. R. WALLACE. (With 12 Text-figures)	107
13.	The pollination requirement of the field bean (*Vicia faba*). By I. B. M. RIEDEL and D. A. WORT (née GIBBONS). (With 1 Text-figure)	121
14.	Topical application of mobile liquids to insects by means of micro-capillary tubes. By P. S. HEWLETT and C. J. LLOYD. (With 3 Text-figures)	125
15.	Some field habitats of mites of stored food products. By D. A. GRIFFITHS. (With 3 Text-figures)	134
16.	Field studies on wheat-bulb fly infestations. By F. RAW	145
17.	*Oscinella frit* L. in south Wales. The relationship between sowing date, plant development and progress of attack in spring oats. By J. H. FIDLER and D. WEBLEY. (With 6 Text-figures)	156
18.	The effect of gibberellic acid on leaf area and dry-matter production in Majestic potato. By E. C. HUMPHRIES and S. A. W. FRENCH. (With 5 Text-figures)	177
Reviews		189

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FIELD TRIALS OF THE EFFECT OF ALDRIN ON CLUBROOT OF SUMMER CABBAGE

BY A. G. CHANNON AND W. G. KEYWORTH

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Field experiments were carried out from 1956 to 1958 on the effect of soil applications of aldrin on clubroot disease of summer cabbage. In the first two seasons the compound was applied either as a dust, broadcast and worked into the soil, or as an emulsion watered round the plants. Results in 1956 were inconclusive and the same plots were used again in 1957, the same quantities of aldrin being re-applied. In this season increasing doses of the compound gave increasing control of the disease. The treatment of individual plants with emulsion was more successful than the application of dusts.

In 1957 half of each plot received a heavy dressing of ground limestone. Lime alone gave a control equal to that given by the highest aldrin treatment. Lime and aldrin together gave a further reduction in clubbing.

In 1958 increasing doses of emulsion were tested. These gave increasing control of disease but the higher doses caused some phytotoxicity which was reflected in a delay in maturation of the crop.

A reduction of clubbing from 'severe' to 'moderate' caused a marked increase in crop, but further reductions in clubbing did not result in any corresponding increase in crop weight.

Several workers have noted that insecticides applied to the soil can affect soil-borne plant pathogens and other micro-organisms (Smith & Wenzel, 1947; Wilson & Choudri, 1948; Cullinan, 1949). Of more immediate application to the present study is the work of Richardson (1957) and Bollen, Morrison & Crowell (1954). The former found that aldrin reduced the root rotting of barley seedlings by *Helminthosporium sativum*, and the latter reported that the same chemical reduced the numbers of fungi in the soil.

The present work arose from observations made during a trial of the effects of soil incorporations of aldrin and BHC on the tainting of vegetables, which was in progress at Luddington Experimental Horticultural Station as an extension of the work of Mr D. W. Wright, Entomology Section, National Vegetable Research Station. Some evidence was obtained from a non-replicated section of this trial that the application of aldrin dust (1·25% aldrin) at the rate of 320 lb./acre (4 lb./acre aldrin) reduced the severity of clubroot on Primo cabbage. In 1956, an experiment to test this further was laid down by the writers at the Luddington Experimental Horticultural Station, with the assistance of its staff, in a field where clubroot was known to be present.

1956 EXPERIMENT

In this experiment there were four randomized blocks, each containing six plots. These plots received the following treatments:

1 and 2. Control (no treatment).
3. Aldrin dust (1·25%)—160 lb./acre (2 lb. aldrin).
4. Aldrin dust (1·25%)—320 lb./acre (4 lb. aldrin).
5. Aldrin dust (1·25%)—640 lb./acre (8 lb. aldrin).
6. Aldrin emulsion (0·04%)—$\frac{1}{8}$ pint per plant (spot treatment).

The dust treatments were variants of that used in 1955. The spot treatment was the one recommended for the control of cabbage-root fly by Wright (1956) and it was included to test whether the commercial use of the treatment for this purpose would also affect clubroot.

The twenty-four plots were each $5\frac{1}{2} \times 6\frac{3}{4}$ yd. separated by $1\frac{1}{2}$ yd. pathways and each contained 120 Primo cabbage plants at a spacing of 27×18 in. Of these, fifty-six plants were guards and sixty-four were recorded.

The cabbages were planted on 9 May, the dusts having been applied to the plots 16 days earlier. The quantity of dust for each plot was mixed with a fixed quantity of sand for ease of application and incorporated to a depth of 3–4 in. by means of a rotavator. Precautions were taken against plot-to-plot contamination by the dusts.

The spot treatment was given on the day after planting, the $\frac{1}{8}$ pint per plant being individually measured. The plots were irrigated 2 days before planting and on three subsequent occasions, a total of 3 in. of water being applied.

The plants were harvested on 18 July. All the plants were cut just below the lowest leaf and the tops (total head and leaf growth) were weighed. The numbers of hearted plants were recorded and the root systems were graded for clubroot on the following scale.

1956 *clubroot grading*

0. No clubroot.
1. Trace. Up to five small clubs; remaining roots fibrous.
2. Slight. Over five small clubs; up to $\frac{1}{4}$ root system affected.
3. Moderate. $\frac{1}{4}$–$\frac{1}{2}$ root system clubbed.
4. Severe. More than $\frac{1}{2}$ root system clubbed.

The results are given in Table 1.

TABLE 1. 1956 *experiment. Mean yields and clubroot grades*

Aldrin application rate	Wt. of tops per plot (lb.)	No. hearted plants per plot	Clubroot index per plant (max. 4)
Nil	79·1	16·2	3·84
2 lb./acre	101·0	31·8	3·82
4 lb./acre	87·7	19·5	3·85
8 lb./acre	110·2	33·0	3·78
$\frac{1}{8}$ pint 0·04% emulsion per plant	107·6	28·5	3·60

Although the clubroot index of the plants receiving the spot treatment was just significantly less than that of the controls, the general indication from this experiment was that aldrin had had very little effect upon the disease. It seemed possible, therefore, that the apparent effect of aldrin in 1955 was caused by the patchy distribution of the disease. Tests indicated, however, that the pH of the soil on the 1955 site was approximately 6·2, whereas that on the 1956 site was approximately 5·4. For this reason it was decided to repeat the experiment in 1957, but, in addition, to test the effect of the aldrin treatments in limed and unlimed soil.

1957 EXPERIMENT

This experiment was made on exactly the same plots as those used in 1956, but half of each plot was given a dressing of carbonate of lime. This was applied to strips running the whole length of each block, the half-block to be limed being chosen at random in each case. Two dressings of ground limestone were given (in early February and early April) at a total rate of 3·5 tons/acre, and these were thoroughly worked into the top 6–8 in. of soil. Seven weeks after the second lime application the pH range on the limed sections was 6·4–7·4 and on the unlimed sections 5·1–6·1. The dusts were re-applied on 16 April at the same rates as in 1956. The cabbages were planted on 2 May, each half-plot containing twenty-eight guard and thirty-two experimental plants. The spot treatment was given on 6 May and was increased from $\frac{1}{8}$ to $\frac{1}{2}$ pint per plant of 0·04% aldrin. The plots were irrigated before planting and during the trial with a total of 3 in. of water.

The first cut of hearted cabbage was taken on 17 July followed by a complete clearing of the plots on 22 July. The root systems were then dug up, washed and graded for clubroot on the following scale, which was considered to be more accurate than the one used in 1956.

1957 clubroot grading

Grade	Proportion of root system clubbed	Notes
0	Nil	
1	Less than $\frac{1}{16}$	Up to five small clubs. Many fibrous roots
2	$\frac{1}{16}$–$\frac{1}{8}$	Two to three medium and several small clubs. Slight reduction in fibrous roots
3	$\frac{1}{8}$–$\frac{1}{4}$	One to two large and several medium clubs. Moderate number of fibrous roots
4	$\frac{1}{4}$–$\frac{1}{2}$	Large and medium clubs coalesced. Few fibrous roots
5	More than $\frac{1}{2}$	Clubs coalesced. Almost no fibrous roots

The results are given in Table 2.

Several plants in the control plots were killed by cabbage-root fly and many were reduced in size. The results for these plots given in the table cannot therefore be used for comparisons of the effect of clubroot on yield. The application of aldrin dust at the rate of 2 lb. aldrin per acre, however, gave good control of root fly (probably because it was supplemented by the residue from a similar dressing in

1956). It can therefore be regarded as the control treatment in respect of clubroot and crop weight assessments.

The results show that, either singly or in association, both the aldrin and the lime had pronounced effects on the disease. On the unlimed plots, increasing quantities of aldrin caused a progressive reduction in clubroot and a corresponding increase in weight of tops. On the limed plots, increasing quantities of aldrin also caused a progressive reduction in clubroot, but this was not associated with a significant increase in the weight of tops. It appeared that where the treatments had suppressed clubroot development below a final grade of 3·3 the disease had little or no effect on plant growth. This was confirmed by a statistical examination of the relationship between clubroot grade and top weight.

TABLE 2. 1957 *experiment. Mean yields and clubroot grades*

Aldrin application rate	Wt. of tops per plot (lb.)	No. hearted plants per plot	Clubroot index per plant (max. 5)
No lime			
Nil	**37·6**	**17·4**	**4·35**
2 lb./acre	53·8	23·5	4·61
4 lb./acre	56·2	23·2	4·16
8 lb./acre	74·6	25·8	3·71
½ pint 0·04 % emulsion per plant	85·9	30·0	3·04
Lime			
Nil	**63·6**	**20·2**	**3·11**
2 lb./acre	82·1	28·0	3·31
4 lb./acre	87·8	29·0	2·66
8 lb./acre	89·8	29·8	2·82
½ pint 0·04 % emulsion per plant	90·4	31·0	1·66
S.E. per plot	9·0	3·0	0·75
L.S.D. between treatment means ($P = 0·05$)	12·9	4·3	1·08

The effect of lime is best shown by a comparison of the clubroot grades and yields on the limed and unlimed plots receiving the lowest dose of aldrin. It is apparent, however, that the high doses of aldrin caused a considerable reduction in disease and a very marked increase in yield even in the absence of lime. It is considered that the difference between the 1956 and the 1957 results on these plots is largely due to the extra aldrin applied in 1957. This, together with that remaining from the previous year, presumably brought the concentration in the soil up to a level which was effective under the conditions prevailing in 1957.

1958 EXPERIMENT

In view of its superior effect in 1957, the spot treatment of plants was investigated further. Greenhouse tests of phytotoxicity to cabbage seedlings made during the winter showed that 0·06 % aldrin applied at a rate of ½ pint per plant caused little damage to plants in pots, but that considerable retardation occurred if the concentration was increased to 0·10 %.

Based on these results, a randomized block experiment was laid down at Ludding-ton using the following rates of aldrin applied to the soil around the stems of Primo cabbages one day after planting.

Series	No.	% aldrin	Amount of liquid applied per plant
A	1	Nil	
	2	0·04	
	3	0·06	½ pint
	4	0·09	
	5	0·135	
B	1	Nil	
	2	0·08	
	3	0·12	¼ pint
	4	0·18	
	5	0·27	

Each of the ten treatments was given to eight single-row plots each containing twenty-five test plants and arranged in randomized blocks. There were single separation rows between the blocks. The cabbage plants were frame-raised and were planted out on 14 May. The aldrin was applied on 15 May, and on 16 May every plant in the experiment (including controls) was given a dressing of a half teaspoonful of 0·45% BHC dust, applied to the soil around the stem base. The purpose of this dressing was to ensure control of cabbage-root fly and thus to avoid the losses from this pest in the control plots, which invalidated the results from such plots in 1957.

No irrigation was necessary during the growth of the crop as the moisture in the soil was adequate throughout the season both for the development of the plants and of the disease.

The plants receiving the higher aldrin doses (A_4, A_5, B_4, B_5) showed symptoms of phytotoxicity during the early stages of growth, being somewhat bluish in appearance and stunted. Once this initial period of retardation was passed, however, the plants made good growth and at harvest time were much larger than the controls.

An assessment of cabbage-root fly damage was made on groups of five plants lifted specially from each plot for this purpose on 4 July. This was done so that such damage could be detected before it had been obscured by clubroot infection. No signs of larvae or mining were observed, although adjoining plants untreated with BHC were severely attacked. It was concluded therefore that there had been complete control of the insect. At this preliminary lifting scarcely any clubroot was observed on plants in A_4, A_5, B_4, B_5, although the disease was developing in the other treatments.

The remaining twenty test plants in each plot were harvested on 28 July, 6 August, and 13 August. On the first two dates, only hearted plants were cut, but on the last date the experiment was cleared.

After each cut the roots were dug up and washed and graded for clubroot on the same scale as that used in 1957.

The accumulated results are shown in Table 3.

TABLE 3. 1958 *experiment. Mean yields and clubroot grades*

Quantity per plant	% aldrin	Wt. of tops per plot (lb.)	No. hearted plants on 28 July (max. 20)	Total no. hearted plants per plot (max. 20)	Clubroot index per plant (max. 5)
½ pint	Nil	35·5	11·6	18·9	4·51
	0·04	60·0	14·5	19·4	3·43
	0·06	68·3	14·9	19·8	3·11
	0·09	63·7	11·8	19·1	2·55
	0·135	61·6	10·4	19·2	2·26
¼ pint	Nil	29·1	10·6	17·4	4·60
	0·08	58·0	15·2	19·8	3·80
	0·12	58·0	13·0	19·2	3·39
	0·18	57·7	12·0	18·8	2·89
	0·27	56·9	8·1	18·4	2·50
	S.E. per plot	7·52	2·46	1·15	0·37
	L.S.D. between treatment means ($P = 0·05$)	7·52	2·46	1·15	0·37

Soil pH readings were made on samples taken to a depth of 6 in. from each block on 13 August. They ranged from 5·20 to 5·85.

The 1958 experiment confirmed the result obtained in the previous year when 0·04% aldrin applied at a rate of ½ pint per plant reduced the amount of clubroot infection. In addition, an increase in the quantities of chemical above this dose caused a progressive reduction in disease.

The differences in crop weight and clubroot index between the plots receiving applications of ¼ and ½ pint aldrin are non-significant. The fact that all of the ¼-pint treatments gave somewhat higher clubroot indices than the corresponding ½-pint treatments suggests, however, that the former may have been somewhat less effective, possibly because of the more restricted soil penetration of the smaller quantity of liquid.

Although the plants receiving the two highest aldrin treatments in each series were free from clubroot on 4 July, they became fairly heavily infected later on. The appearance of the roots suggested that this infection occurred when the roots grew beyond the treated zone.

All the treatments caused a considerable and highly significant increase in yield, confirming the results obtained in 1957. With the exception of the 0·06% treatment, increasing the dose of aldrin did not result in any significant increase in yield—a fact which is presumably a reflexion of the ability of the cabbage plant to tolerate fairly heavy infection of its root system, as noted in 1957. There was some evidence of a reduction in yield at the higher levels of aldrin—probably due to phytotoxicity—but this was not statistically significant.

The effect of phytotoxicity was markedly shown in the numbers of hearted plants cut on 28 July, when it was quite obvious that the higher aldrin treatments had greatly retarded the crop by comparison with the lower doses.

The evidence obtained from the 3 years trials shows that aldrin will reduce clubroot, especially when applied as a liquid 'spot treatment'. It is also apparent, however, that excessive doses may cause phytotoxicity with retardation and possibly reduction of the crop.

The writers wish to thank the Director of Luddington Experimental Horticultural Station for permission to carry out these experiments and the staff for their very considerable assistance during the work.

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STUDIES OF THE BIOLOGY OF *SCLEROTIUM CEPIVORUM* BERK.

IV. GERMINATION OF SCLEROTIA

By J. R. COLEY-SMITH*

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(With Plate 1 and 2 Text-figures)

Sclerotia from 6-week-old pure cultures of *Sclerotium cepivorum* germinated immediately in soil only after abrasion of their rinds, but after burial in soil for a month or more, unabraded sclerotia became capable of germination.

Marked stimulation of germination occurred in the presence of host plants (onion, leek and shallot). Little or no germination occurred in soil alone or in the presence of non-host plants (barley, cabbage, carrot and white clover). Sclerotial germination was observed in a number of soils of widely differing pH and over a wide range of soil water content. Germination of sclerotia on uninjured onion roots was greatest at the tip region. On artificially injured roots sclerotial germination was enhanced but the effect of position was lost.

Sclerotial germination was independent of contact between roots and sclerotia. It was induced by root extracts of all *Allium* spp. tested, but of no other plants. Boiling or autoclaving root extracts did not destroy the active principle and it is concluded that under field conditions sclerotia are induced to germinate by a thermostable chemical substance from *Allium* roots.

The process of germination of sclerotia is described.

Introduction

In a previous paper (Coley-Smith, 1959) sclerotia of *Sclerotium cepivorum* Berk. were shown to persist in soil under field conditions, for periods of up to 4 years. However, in order to function satisfactorily as perennating bodies sclerotia must not only be capable of persistence in the absence of host plants, but must also be able to germinate and infect the host, under appropriate circumstances. Scott (1954) discovered that sclerotia from 6-week-old pure cultures would germinate in soil only after artificial abrasion of their rinds. Coley-Smith & Hickman (1957) reported that the presence of onions in soil stimulated germination of suitably aged sclerotia and suggested that the effect was a chemical one. The present paper describes further experiments on the influence of the host and its extracts on sclerotial germination.

Materials and methods

The isolate of *Sclerotium cepivorum* and method of production of sclerotia were those used previously (Coley-Smith, 1959). Unless stated otherwise, sclerotia were buried for 6 months in soil in a glasshouse before use. In some experiments

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sclerotial germination was measured indirectly, as before, by counting infected seedlings but in the majority of experiments direct observations were made of germination on a 10 × 2·5 cm. strip of nylon material (average mesh size 128 μ) placed inside a 4 × 1$\frac{1}{4}$ in. glass specimen tube, near the top, and moistened so that the nylon stuck to the glass. Sclerotia were then placed with forceps in a circle between the nylon and the side of the tube. To examine the effect of seedling onions (var. White Lisbon) and other plants on sclerotial germination, seeds were placed in another circle 1 cm. above the sclerotia. After addition of soil both sclerotia and seeds remained in position and the tubes were incubated at a constant water content (Keen & Raczkowski, 1921). Black paper secured around the outside of the tubes discouraged the growth of algae.

In the field, a glass tube 12 × 1 in. was used, permitting root growth over a long period. The bottom of the tube was plugged with a nylon-covered cork, pierced with a 1 cm. diameter hole which was plugged with soil. This allowed free drainage yet prevented growth of roots into the surrounding soil and hence their injury during removal of the tube for examination. Sclerotia were placed on a strip of nylon near the top of the tube and onion seeds grouped similarly 1 cm. higher up. The tubes were then filled with soil and buried up to their rims under natural conditions in the field.

Experimental results

In the early part of this work Scott's (1954) statement, that sclerotia from 6-week-old cultures would germinate in soil only after their rinds had been artificially damaged by abrasion, was confirmed on numerous occasions. Sclerotia which were ground gently with a pestle and mortar until their rinds were broken germinated readily on nylon strips in soil tubes, whereas undamaged ones did not. On agar media sclerotia of all ages germinated equally readily, without injury.

In tests using single, abraded sclerotia low levels of infection always resulted but infection was raised by increasing the number of abraded sclerotia per onion seedling. However, when unabraded sclerotia which had been buried in the field for 2$\frac{1}{2}$ years were used singly as inocula very high levels of infection occurred. To find how long a period in soil was necessary before unabraded sclerotia became infective, the following experiment was made.

After inoculation with 6-week-old sclerotia, which had been buried in pots of soil under glasshouse conditions for periods of from 1 to 6 months, White Lisbon seedlings were placed in the laboratory (60–70° F.) in tubes of Edgbaston garden loam (pH 7·0) at 40% water-holding capacity (hereafter referred to as w.h.c.). Records of infection are shown in Table 1. No further infection occurred after 8 weeks.

Sclerotial dormancy started to break down shortly after burial in soil and after 2 months it had almost, if not completely, disappeared. This result has been confirmed with sclerotia buried under field conditions.

In the above experiment, and in others, high levels of germination (usually 60–92%) were obtained by the use of the infection technique with suitably aged

sclerotia. On the other hand germination of sclerotia on nylon strips in soil tubes, without onions present, was usually poor (0–15%). This suggested a stimulatory effect of the host on sclerotial germination and the following three nylon strip experiments were designed to examine this possibility. In each of these experiments there were three tubes per treatment, each with twelve sclerotia and twelve seeds. Replicate values have been summed for convenience of presentation. No germination of sclerotia occurred in control tubes without seeds and these have been omitted from the tables of results.

TABLE 1. *Infection of White Lisbon onion seedlings by* Sclerotium cepivorum *sclerotia buried beforehand for various periods in soil*

Duration of previous burial of sclerotia in months	No. of seedlings	No. of seedlings infected Weeks 1	2	3	4	5	6	7	8
0*	14	0	0	0	0	0	0	0	0
1	14	0	0	1	2	2	3	3	5
2	14	0	1	2	3	9	11	11	11
3	14	0	3	8	9	10	13	13	13
4	14	0	1	5	7	8	11	11	12
5	14	0	1	6	10	11	12	12	12
6	14	0	3	10	11	12	12	12	12

* Direct from culture.

TABLE 2. *Germination of 6-month-old sclerotia of* Sclerotium cepivorum *in Edgbaston garden loam in the presence of onions, leeks and shallots*

Plant	% water-holding capacity of soil	No. of seeds germinated	No. of sclerotia germinated Weeks 1	2	3	4	5	6	7	8	Final % sclerotial germination	No. of seedlings infected by white rot	Infection index
Onion	40	26	0	0	0	0	1	4	6	6	16·7	23	1·44
	60	26	0	0	0	1	4	9	10	10	27·8		
Leek	40	26	0	0	1	4	5	7	9	9	25·0	3	0·16
	60	25	0	1	1	2	3	6	8	10	27·8		
Shallot	40	25	0	0	0	5	6	7	9	9	25·0	8	0·33
	60	25	0	1	5	9	10	13	15	15	41·7		

In the first of these experiments sclerotia in tubes of Edgbaston garden loam (pH 6·5) were maintained at 40 or 60% w.h.c. with distilled water. Seeds of the following plants were used: onion var. White Lisbon, leek var. Musselburgh, shallot var. Giant Red, barley var. Atlas 46, cabbage var. Harbinger, carrot var. Early Horn, white clover var. S100. No germination of sclerotia occurred in the presence of the last four plants and these are excluded from the results shown in Table 2.

Although there was a marked effect of the presence of *Allium* seedlings on sclerotial germination there was little difference in the stimulatory capacities of onions, leeks, and shallots. An examination of the infection index (number of seedlings infected with white rot divided by number of sclerotia germinated) shows that stimulatory

capacity and susceptibility to white rot are not necessarily related. Although onions caused slightly less sclerotial germination than leeks the infection index for onions was nine times as great as that for leeks. Mycelium from germinating sclerotia persisted for about a fortnight and then disappeared, leaving the sclerotial rinds as broken, hollow shells which themselves ultimately decayed.

Stimulation of sclerotial germination by onion seedlings was also examined in soils of widely differing pH:

(1) Sutton Park podsol, average pH 3·5.

(2) King's Norton loam. A heavy clay loam; average pH 4·8.

(3) Edgbaston garden loam. A lighter, more sandy loam; average pH 6·0.

(4) Edgbaston garden loam to which lime (CaO) was added. The pH at the beginning of the experiment was 8·5, dropping to 7·2 by the end.

In the podsol soil, seed germination and subsequent seedling growth was very poor and there was a vigorous development of *Trichoderma viride* on both seeds and nylon strips. After a month all the seedlings had died and no sclerotia germinated. Results for this series are therefore excluded from the results in Table 3.

TABLE 3. *Germination of 6-month-old sclerotia of* Sclerotium cepivorum *in the presence of White Lisbon onions in various soils*

Soil	% water-holding capacity	No. of onion seeds germinated	No. of sclerotia germinated Weeks 1	2	3	4	5	6	7	8	Final % sclerotial germination
King's Norton loam	40	25	0	0	1	2	3	7	7	9	25·0
	60	32	0	0	1	2	5	5	5	7	19·4
Edgbaston garden loam	40	23	0	0	0	2	5	10	17	22	61·1
	60	29	0	2	4	5	8	9	17	25	69·4
Edgbaston garden loam plus lime	40	21	0	0	9	22	28	31	35	35	97·2
	60	29	0	0	7	23	28	30	33	34	94·4

There were very large differences in the levels of sclerotial germination in the three soils, the greatest germination occurring at the highest pH. This relationship has been confirmed with a natural, alkaline soil, Lias clay (average pH 7·6) in which germination was greater than in Edgbaston garden loam or King's Norton loam.

To study the effect of soil moisture on sclerotial germination, tubes containing Edgbaston garden loam (pH 7·0) adjusted to 20–100% w.h.c. were set up. Germination of onion seed was satisfactory over the whole range except at the 100% level, where it was very poor. Results for this water level are consequently not included in the results (Table 4).

There was a general rise in sclerotial germination with increasing soil moisture content. On the other hand the pattern of onion seedling infection was very different, the infection index being highest at moderate water levels with a tendency to fall off at both extremes of the range.

Since the presence of the host had such a marked stimulatory effect on sclerotial germination in the laboratory, attempts were made to discover whether similar effects occurred in the field.

Eighteen glass germination tubes as described earlier, each with ten sclerotia and filled with Edgbaston garden loam (pH 6·9), were set up. In six of these eight White Lisbon seeds were grouped in the usual manner. In another six tubes a single seedling at the second true-leaf stage was planted in the centre of each, and the remaining six were left without onion seeds as controls. The tubes were buried up to their rims in the field on 1 April 1958. Apart from periodic examinations they were left under completely natural conditions. A record of sclerotial germination is shown in Table 5. Tubes without onions are not included since no germination occurred in them. In the other two treatments replicate values have been summed.

TABLE 4. *Germination of 6-month-old sclerotia of* Sclerotium cepivorum *in the presence of White Lisbon onions, in Egbaston garden loam adjusted to different moisture levels*

% water-holding capacity	No. of onion seeds germinated	No. of sclerotia germinated Weeks 1	2	3	4	5	6	7	8	Final % sclerotial germination	No. of seedlings infected by white rot	Infection index
20	32	0	0	0	1	2	3	3	3	8·3	1	0·33
30	31	0	0	0	0	2	4	4	4	11·1	3	0·75
40	33	0	0	1	3	3	5	6	6	16·7	12	2·00
50	33	0	0	0	0	1	6	8	8	22·2	15	1·88
60	28	0	0	0	5	7	10	11	13	36·1	12	0·92
70	33	0	0	0	0	3	7	11	11	30·6	15	1·36
80	31	0	0	2	7	11	16	17	17	47·3	22	1·29
90	26	0	0	3	16	19	26	27	27	75·1	20	0·74

TABLE 5. *Germination of 6-month-old sclerotia of* Sclerotium cepivorum *in tubes of Edgbaston garden loam, in the field, in the presence of White Lisbon onions*

Treatment	No. of sclerotia germinated 18 Apr.	28 Apr.	13 May	21 May	27 May	2 June	9 June	16 June	23 June	3 July	7 July	21 July	11 Aug.	Final % sclerotial germination
8 onion seedlings per tube	0	0	2	13	28	37	44*	—	—	—	—	—	—	73·3
1 onion seedling per tube	0	0	0	1	1	1	2	5	6	7	13	14	28	46·7

* Tubes abandoned, all onion seedlings dead.

In the series with eight onions per tube 56·7% of the seedlings became infected with white rot, and in the treatment with a single onion per tube two of the six seedlings were infected. This experiment proved that stimulation of sclerotial germination followed by infection of onion seedlings can occur under field conditions.

A criticism which could be applied to the nylon-strip method for studying sclerotial germination is that it does not simulate fully the conditions which sclerotia would encounter in nature completely surrounded by soil. An experiment was therefore devised to discover whether onions have any effect on sclerotia dispersed naturally in soil.

One hundred sclerotia were mixed in each of a number of $4 \times 1\frac{1}{4}$ in. glass specimen tubes containing Edgbaston garden loam (pH 6·9). Two types of sclerotia were used:

(1) From a 6-week-old sand corn-meal culture.

(2) From 6 months burial in soil.

Ten onion seeds were sown in half of the tubes and were replaced with fresh ones when seedlings infected with white rot were observed. All tubes were kept under laboratory conditions at 40% w.h.c. Sclerotia were recovered by the sieving method described previously (Coley-Smith, 1959). The final recovery figures after 1 year are shown in Table 6.

TABLE 6. *Persistence of sclerotia of* Sclerotium cepivorum *in Edgbaston garden loam in the presence or absence of White Lisbon onions*

Age of sclerotia	With (+) or without (−) onions	No. of sclerotia recovered in three replicates			Average % recovery of sclerotia
6 weeks	+	51	68	27	48·7
	−	100	95	96	97·0
6 months	+	72	8	17	32·3
	−	94	98	88	93·3

Despite the large variation between replicates there was a marked effect of the host on sclerotial numbers and this was obviously due to stimulated germination. Frequent infection of onion seedlings occurred and in a few instances sclerotia were observed in a germinating condition during recovery.

In the foregoing experiments it was often noted that sclerotial germination was enhanced in the vicinity of decaying seedlings, which suggested that host injury might be important. An experiment was designed to examine this possibility.

White Lisbon seeds were grown for 5 days at 20° C. in Petri dishes lined with moist filter-paper. Sclerotia from 6-months soil burial were placed on the roots of the seedlings in the following positions:

(1) On the root tip.

(2) Midway between the root tip and the developing bulb region.

(3) On the developing bulb region.

In half of the dishes seedlings were uninjured and in the remainder the root surfaces were punctured with a needle, sclerotia then being placed on the wounded area. There were forty sclerotia per treatment, twenty in each of duplicate dishes. They were transferred to fresh seedlings every 5 days and examined microscopically for germination. Results are shown in Table 7, duplicate values being summed. No germination occurred of control sclerotia placed on moist filter-paper.

On uninjured roots there was a very marked positional effect, sclerotial germination being greatest at the root-tip region. Root injury whilst bringing about an increase in germination destroyed the positional effect.

Another phenomenon occurring in the soil experiments described was the frequent germination of sclerotia at distances of up to a centimetre from the

nearest host root. This suggested a chemical stimulus rather than a contact effect. Experiments were designed to investigate this point more fully and to provide further information on the nature of the stimulus involved.

Sclerotia were placed on moist filter-paper in Petri dishes. In half of the dishes they were positioned inside glass rings (1 in. diameter) resting on the surface in the centre of the dishes, and in the remainder on filter-paper in subsidiary dishes enclosed within the Petri dishes. White Lisbon onion seeds (1·5 g.) were scattered around the outer portion of half of the dishes, contact with sclerotia being prevented

TABLE 7. *Germination of 6-month-old sclerotia of* Sclerotium cepivorum *at 20° C. on roots of White Lisbon onion seedlings*

Injured or uninjured roots	Position of sclerotia on root	No. of sclerotia germinated Weeks 1	2	3	4	5	6	7	8	Final % sclerotial germination
Uninjured	Root tip	5	21	29	38	39	39	39	39	97·5
	Between tip and bulb	3	3	16	16	17	18	19	19	47·5
	On developing bulb region	0	6	15	18	19	20	21	23	57·5
Injured	Root tip	19	37	40	—	—	—	—	—	100
	Between tip and bulb	19	39	40	—	—	—	—	—	100
	On developing bulb region	23	39	40	—	—	—	—	—	100

TABLE 8. *Germination of 6-month-old sclerotia of* Sclerotium cepivorum *at 20° C. on moist filter-paper in the presence or absence of White Lisbon onion seedlings*

With (+) or without (−) onions	Sclerotia on filter-paper within glass ring in Petri dishes (A) or in subsidiary dish within Petri dish (B)	Total number of sclerotia germinated (out of 240) after 14 days incubation
+	A	74
	B	12
−	A	2
	B	1

either by the glass rings or the subsidiary dishes. In the former case stimulation could occur by diffusion of the stimulant through the filter-paper, but the sclerotia in subsidiary dishes could only be stimulated if the active principle were volatile. Dishes were incubated at 20° C. and were kept moist with distilled water. Non-viable seeds were removed daily to prevent excessive contamination of the dishes by saprophytic moulds. Three experiments of this type were carried out, in each of which there were four dishes per treatment with twenty sclerotia per dish. The experiments were performed under identical conditions and the final germination figures, recorded after 14 days incubation, have been summed in Table 8.

The results show that contact between sclerotia and host roots is not necessary for stimulation of sclerotial germination. The active principle was apparently able to pass quite readily through moist filter-paper but was only slightly if at all volatile.

Since contact with host roots is not necessary for stimulation of sclerotial germination it should occur in the presence of root extracts or exudates from living roots. This possibility was examined by treating sclerotia with root extracts of various plants.

Root extracts were made from 6-week-old seedlings by grinding 10 g. fresh root tissue to pulp, suspending in 250 ml. distilled water, and filtering off the debris. The following plants were used: onion var. White Lisbon, leek var. Musselburgh, shallot var. Giant Red, cabbage var. Harbinger, and brussels sprout var. Cambridge no. 5. Nylon-strip germination tubes were set up, filled with Edgbaston garden loam (pH 7·0) and adjusted to 40% w.h.c. with distilled water. They were incubated under laboratory conditions and the original water levels maintained by the addition every 2 days of the appropriate root extract (approximately 2 ml.). There were three tubes per treatment, each with ten sclerotia. No germination of sclerotia occurred in the distilled-water controls or in those tubes which received brussels sprout or cabbage extracts. It was entirely confined to the tubes which were watered with *Allium* extracts, where germination figures were consistently high. Summed values of replicates are shown in Table 9.

TABLE 9. *Germination of 6-month-old sclerotia of* Sclerotium cepivorum *in Edgbaston garden loam watered with root extracts of onion, leek and shallot*

	No. of sclerotia germinated Weeks								Final % sclerotial
Extract	1	2	3	4	5	6	7	8	germination
Onion	0	0	3	8	15	20	23	26	86·7
Leek	0	0	2	6	8	14	20	22	73·3
Shallot	0	0	4	11	13	21	26	28	93·3

TABLE 10. *Germination of 6-month-old sclerotia of* Sclerotium cepivorum *in Edgbaston garden loam watered with root extracts of* Allium *spp.*

	No. of sclerotia germinated Weeks								Final % sclerotial
Extract	1	2	3	4	5	6	7	8	germination
Onion	0	0	0	0	1	5	7	7	23·3
Crow garlic	0	0	9	11	12	16	22	22	73·3
Garlic	0	0	5	7	20	23	24	24	80·0
Ramsons	0	0	1	2	10	24	26	26	86·7

In another experiment, set up in the same way, root extracts of the following plants were used: beetroot var. Crimson Globe, carrot var. Early Horn, barley var. Atlas 46, onion var. White Lisbon, crow garlic (*Allium vineale*), garlic (unknown variety), ramsons (*A. ursinum*), and garlic mustard (*Alliaria petiolata*). The first four of these were 6-week-old seedlings, and the remainder were plants of unknown age from the field. Again germination of sclerotia was confined to those tubes watered with extracts of *Allium* spp. (Table 10), with the exception of a single sclerotium in a tube which received garlic mustard extract. No explanation can be given for the low levels of germination with onion extract.

In the final experiment to be described the effect of boiling and autoclaving root extracts was investigated.

The extract was made from roots of 1-month-old White Lisbon seedlings by grinding 20 g. fresh tissue to pulp, extracting with distilled water and making up to 500 ml. The extract was divided into five 100 ml. portions which were treated as follows:

(1) Untreated.
(2) Seitz-filtered.
(3) Autoclaved for 15 min. at 15 lb./in.^{2} pressure.
(4) Boiled for 5 min.
(5) Boiled for 15 min.

Table 11. *Germination of 6-month-old sclerotia of* Sclerotium cepivorum *in Edgbaston garden loam* (pH 7·2) *watered with White Lisbon root extract subjected to various treatments*

Treatment	No. of sclerotia germinated Weeks 1	2	3	4	Final % sclerotial germination
Untreated extract	0	1	13	23	76·7
Seitz-filtered extract	0	0	11	14	46·7
Autoclaved extract	0	1	17	20	66·7
Extract boiled 5 min.	0	1	14	18	60·0
Extract boiled 15 min.	0	3	15	23	76·7

After treatment volumes were restored with distilled water. The extracts were added to sclerotial germination tubes every 2 days to maintain these at 40% w.h.c. There were three tubes per treatment, each with ten sclerotia. Records of sclerotial germination are shown in Table 11, replicate values having been summed.

None of these treatments appeared to have had any very marked effect on the potency of the extract and it is presumed that the active principle is thermostable.

Process of germination

As the process of germination of sclerotia of *Sclerotium cepivorum* has not before been described, sclerotia for anatomical study were obtained from the foregoing germination experiments. They were either mounted in water for microscopical examination or fixed, dehydrated, embedded and sectioned.

Mature sclerotia of *S. cepivorum* possessed an outer rind of one to two layers of much-thickened, heavily pigmented rounded cells, enclosing a large medullary region consisting of fairly closely packed elongated hyphae.

The first external sign of germination was the appearance of a bulge on the sclerotial surface. After this the rind was ruptured, presumably by pressure from within, and a large dense plug of mycelium was extruded (Fig. 1 A, B, C). In some of the root-extract germination experiments described previously, especially in the presence of *Allium sativum*, *A. vineale*, and *A. ursinum* extracts, as many as three large plugs were formed by a single sclerotium. The hyphal plug originated from the medullary region, apparently by elongation of already existing cells.

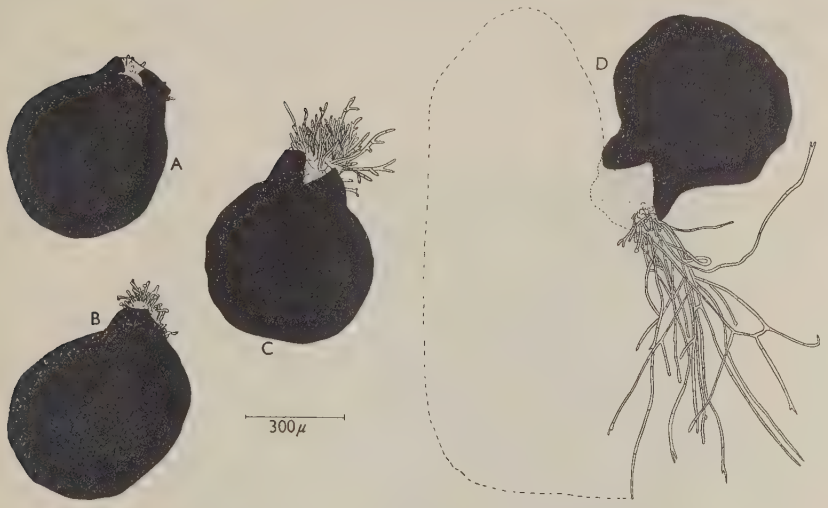

Fig. 1. Stages in germination of *S. cepivorum* sclerotia, from nylon strips in soil tubes, showing rupture of the rind A, followed by extrusion of a plug of mycelium B, C, D.

Fig. 2. Stages in germination of *S. cepivorum* sclerotia, from nylon strips in soil tubes. A, development of microconidia on exposed medullary region of sclerotium; B, early stages in sclerotial initial formation; C, D, later stages in formation of sclerotial initials; E, sclerotial initials and sporodochia with microconidia and a tuft of microconidia on a short side branch; F, microconidia, borne in verticils; G, phialides with microconidia borne direct upon a hypha.

Shortly after emergence of the sclerotial plug, hyphae grew out from the latter and anastomosed freely (Fig. 1 D). Ultimately the hyphae grew to distances of up to several millimetres from the sclerotial body. Frequently the latter was torn apart by the force exerted by the emerging hyphae, thus exposing the central medullary region, which often became covered with tufts of microconidia (Fig. 2 A).

Two or three days after commencement of germination, sclerotial initials were produced at the tips of many hyphae (Fig. 2 B, C, D, E) as described from agar cultures by Townsend & Willetts (1954). Only on rare occasions were sclerotial initials ever observed to form mature sclerotia, although coalescence of small initials to form larger ones was quite common.

In addition to sclerotial initials microconidia were also produced on the germination hyphae. These were very small (1·6–2·0 μ in diameter) and were borne in chains on flask-shaped phialides which occurred in verticils (Fig. 2 F), on short side branches (Fig. 2 E), or in tufts directly upon a hypha. Sometimes an actual cushion or sporodochium was formed on which microconidia were borne in vast numbers (Fig. 2 E). Microconidia were never observed to germinate and their function, if any, is unknown.

Dissolution of the hyphae and sclerotial initials occurred some 10–12 days after germination and ultimately the sclerotia were left as broken hollow shells, the central medullary region having disappeared entirely.

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EXPLANATION OF PLATE 1

A sclerotium of *Sclerotium cepivorum* Berk. germinating on a nylon strip near an onion root.

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COLEY-SMITH—*Biology of* Sclerotium cepivorum *Berk. IV*

(*Facing p.* 18)

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THE INCIDENCE OF CERTAIN SEED-BORNE DISEASES IN COMMERCIAL SEED SAMPLES

II. ERGOT, *CLAVICEPS PURPUREA* (FR.) TUL., IN CEREALS

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(With 1 Text-figure)

The incidence of ergot, *Claviceps purpurea* (Fr.) Tul., in commercial cereal seed samples submitted to the Official Seed Testing Station from 1918 to 1957 has been examined.

Rye samples were more frequently contaminated than wheat or barley and no cereal ergots were recorded in oats.

A relatively high proportion of ergot-contaminated rye samples occurred every 8–10 years. This appeared to be associated with high relative humidity and low maximum temperature during June. Similar trends were found for wheat and barley.

No varietal differences in susceptibility to ergot contamination were found for rye or barley, but there was some evidence that spring wheat varieties were more frequently contaminated than winter ones.

Introduction

In the United Kingdom, ergot, *Claviceps purpurea* (Fr.) Tul., is a frequent contaminant of cereal seed samples, particularly rye. It is not an important disease but occurs to a varying extent in all years. The incidence of ergot contamination in samples of rye received by the Official Seed Testing Station for England and Wales has been reported each year since 1952 in the *Rep. nat. Inst. agric. Bot.* (Annual Report) and before that in the Report of the O.S.T.S. which was included in the *J. nat. Inst. agric. Bot.* (1922–50). The incidence of ergots in these rye samples was reviewed by Eastham (1931–4), and later Dillon Weston & Taylor (1942) discussed the incidence of ergots in rye, wheat and barley from 1918 to 1940. In 1956 reports from farmers and seed merchants suggested that the disease was becoming more common and therefore the records of the incidence of ergots were re-examined and the possibility of varietal differences was investigated. The results of this survey are given below.

Origin of data

When ergots are present in cereal seed samples submitted to the Official Seed Testing Station for statutory tests they are detected with the naked eye during routine purity analyses. Usually no statement of the weight of ergot present is made, and both broken and whole ergots are noted on the official report.

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OBSERVATIONS ON DATA

The incidence of ergot-contaminated samples in rye, wheat and barley samples submitted for routine tests is shown in Fig. 1. The numbers of samples examined each year have increased during the years of the survey from 112 to 379 rye samples, from 2423 to 9170 wheat samples and from 1543 to 5535 barley samples.

The incidence of ergot-contaminated rye samples

Ergots were frequently recorded in rye seed samples and within the 40 years of the survey there have been five periods, each lasting 2–3 years, during which a very high proportion of samples were contaminated (Fig. 1). The seasons of highest infection were 1918–20, 1927–8, 1936–9, 1945–8, and 1954–6; this suggests an 8- to 10-year cycle.

Infection is known to occur during anthesis of the florets (June) and to be affected by humidity and temperature. Nearly all the rye grown in the United Kingdom is autumn sown on the poor acid soils of the drier districts of England, particularly of East Anglia. The meteorological records for Cambridge (Botanic Gardens) were studied as being representative of this eastern area and also because this station gives the longest run of records. The June monthly relative humidity at 9 a.m. G.M.T. and the average daily maximum air temperature were examined (Fig. 1). (The 9 a.m. figures were the only ones available from 1921 but the 3 p.m. records for Mildenhall, Suffolk, give similar figures for 1936–57.) Years in which the percentage of ergot-contaminated samples decreased or remained low tended to be years with records of low humidity (70% or less) or of high temperature (70° F. or more). The biggest reduction in contamination occurred when these conditions were recorded in the same year, e.g. 1921–2, 1940, 1949 and 1957. The years in which ergot-contamination increased or was high tended to be those when humidity was high (74% or more) or temperature was low (66° F. or less) and rapid increase in ergot contamination took place when these conditions occurred in the same year, e.g. 1923, 1926 and 1946. In recent years distinct varieties of rye have been developed but no indication of varietal susceptibility to ergot contamination was detected. Dillon Weston & Taylor (1942) reported that more ergot-contaminated samples were received from Yorkshire than from East Anglia in the three seasons 1939–42 but in the samples received in 1957 such a distribution was not apparent.

The incidence of ergot-contaminated wheat samples

There was a lower ergot contamination in wheat than rye samples and it was always 2% or less (except 2·2 and 2·4% in 1931 and 1932, respectively).

A periodic fluctuation of contaminated samples, similar to rye, was apparent. Highest contamination occurred in 1926–7, 1931–3, 1936–7, 1945, 1947 and 1953–7.

Table 1 shows that in any year the spring wheats tended to be more frequently contaminated than the winter wheats, e.g. in 1956 all six and in 1957 five out of these six spring varieties produced ergot-contaminated samples compared with only

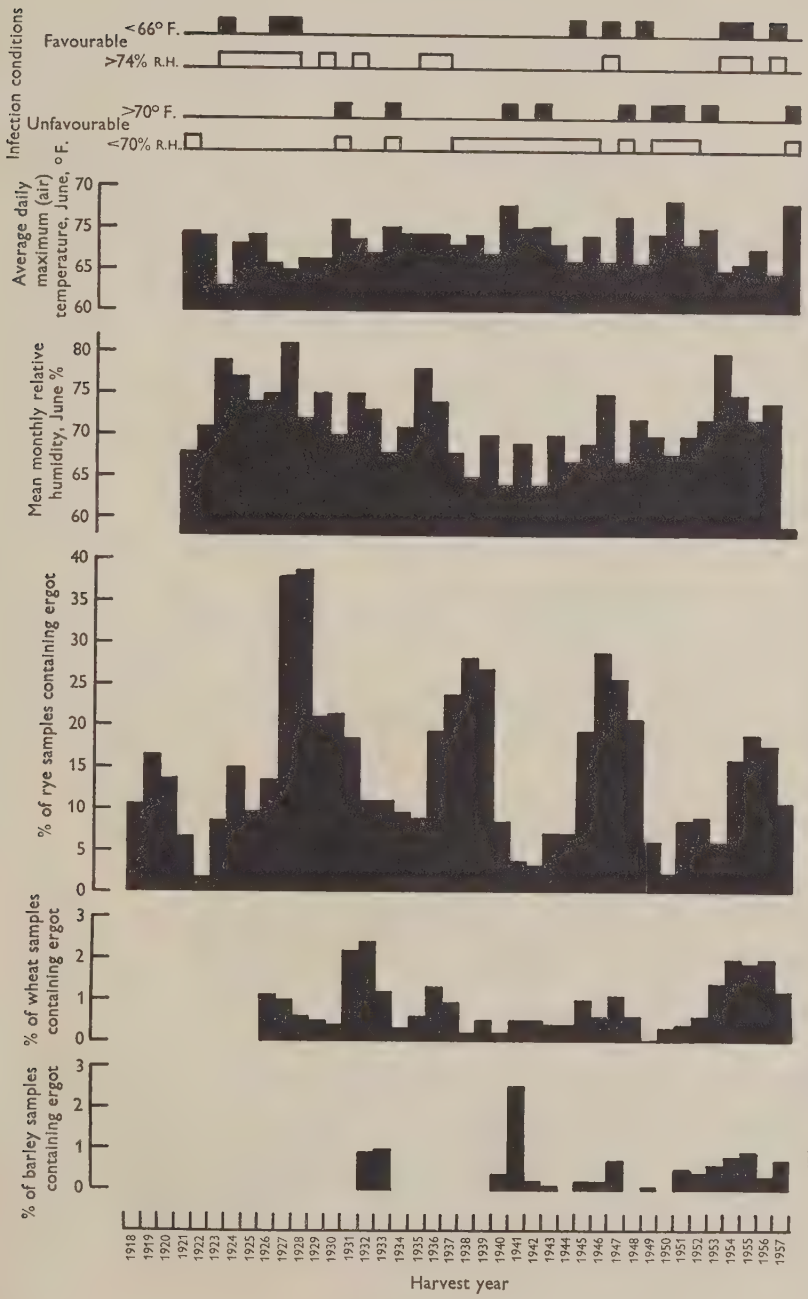

Fig. 1. The incidence of ergot-contaminated samples of rye, wheat and barley in relation to associated weather.

TABLE 1. *Incidence of ergot-contaminated samples in some varieties of wheat.* 1954–7

Harvest year ...	1954		1955		1956		1957		1954–7
Variety	No. of samples examined	Percentage of ergot-contaminated samples	No. of samples examined	Percentage of ergot-contaminated samples	No. of samples examined	Percentage of ergot-contaminated samples	No. of samples examined	Percentage of ergot-contaminated samples	Average percentage of ergot-contaminated samples
Spring varieties									
Koga II	—	—	879	3·6	2894	2·9	1855	2·5	2·9
Atson	29	6·9	476	3·8	956	2·6	314	1·6	2·8
Svenno	—	—	11	0	425	1·4	714	2·2	1·9
Peko	—	—	2214	1·9	879	1·6	327	1·2	1·8
Atle	1513	1·7	1702	1·6	1833	1·6	617	2·1	1·7
Bersee	256	2·7	387	0·8	155	1·9	72	0	1·5
Mean of spring varieties	—	1·9	—	2·2	—	2·3	—	2·2	2·2
Winter varieties									
Minister	273	3·3	273	1·8	126	0	77	0	1·9
N.59	182	1·6	149	2·7	114	0	121	0	1·4
Banco	—	—	183	1·6	485	1·2	262	1·5	1·4
Eclipse	173	1·7	118	0	65	3·1	28	0	1·3
Cappelle Desprez	1380	1·6	2147	1·2	4619	1·4	2802	0·4	1·1
Hybrid 46	841	1·1	702	0·6	890	0	625	0·6	0·6
Heines VII	—	—	792	0·6	606	0·7	266	0·4	0·6
Wilma	174	0·6	227	0·4	123	0	112	0	0·3
Mean of winter varieties	—	1·6	—	1·0	—	1·1	—	0·4	1·0

four out of the eight winter varieties. In general, the highest percentage of ergot-contaminated samples occurred in 1954, but the varieties introduced in 1955 tended to show heavier contamination than these older ones.

The incidence of ergot-contaminated barley samples

Ergot-contaminated barley samples only once exceeded 1% (2·5% in 1941) and in some years no contaminated samples were recorded (Fig. 1). Fluctuation in contamination was again less obvious than for rye but maximum percentages were recorded in 1932–3, 1941, 1947, 1954–5 which, except for 1941, corresponds with wheat infection maxima.

In 1956 and 1957 there was no difference between the incidence of ergot-contaminated samples of twelve common barley varieties (Table 2).

TABLE 2. *Incidence of ergot-contaminated samples in some varieties of barley. 1956 and 1957*

Harvest year ...	1956		1957	
	No. of samples examined	No. of ergot-contaminated samples	No. of samples examined	No. of ergot-contaminated samples
Scandinavian-bred varieties				
Herta	1322	4	722	9
Rika	312	1	404	3
Freja	236	1	220*	0
Carlsberg	265	1	150*	0
Carlsberg II	35	0	30*	0
English-bred varieties				
Proctor	3547	9	2316	14
Earl	386	1	300*	0
Pioneer	282	1	435	1
Maythorpe	199	1	110*	0
Plumage-Archer	153	1	80*	0
Spratt-Archer	107	1	51	1
Provost	162	0	80*	0

* Approximate number of samples examined.

The incidence of ergot-contaminated oat samples

Ergots were only occasionally found in seed oat samples and were always grass ergots.

Discussion

Since 1918 four of the five periods of high ergot contamination of rye seed samples have been closely associated with years in which high average relative humidity (74% or more) and low maximum temperature (66° F. or less) were recorded in June (Fig. 1). They tended to end suddenly with records of low humidity (less than 70%) and high temperature (70° F. or more), e.g. 1940, 1949 and 1957. However, the peak infection years 1936–9 do not show such a close association with recorded weather and in some years, in spite of apparently favourable weather conditions,

the percentage of contamination decreased rather than increased as might have been expected, e.g. 1929 and 1953. Other factors therefore play a part in the determination of the percentage contamination. The size of the inoculum potential in the previous year and perhaps also the local weather conditions, which tend to be obscured in average figures, are probably important. These factors may also help to explain the delay in the change of the percentage of ergot-contaminated samples after favourable or unfavourable weather conditions (e.g. 1925, 1930 and 1941).

Vladimirsky (1939) found that the maximum development of ergots in rye occurred in regions of the U.S.S.R. where there was a high relative humidity (not less than 74%), a temperature at the beginning of flowering of not more than 13–15° C. (54–59° F.) and a prolonged flowering period of 14 days. The present survey also shows that a relative humidity of more than 74% tended to encourage ergot contamination. However, the average maximum temperatures were all higher than 54–59° F., but because they were *average* figures there must have been periods during which temperatures were below this level. It is significant therefore that the biggest effect of temperature was noted in years with a very high maximum temperature. The length of the anthesis period is influenced by humidity and temperature and is longest in wet cool conditions which also favour infection. The survey therefore emphasizes the importance of humidity and temperature and shows that an annual rather than a geographical variation in weather conditions can influence the occurrence of ergot.

Seasonal fluctuation in ergot contamination was also detectable in wheat and barley and agreed closely with that found in rye, except for barley in 1940. While rye is open-flowered and largely cross-pollinated the flowers of wheat and barley are open for only a very short time so that the chance of infection is much less. Also wheat and barley, unlike rye, are more widely grown in the United Kingdom (for which the meteorological data quoted in Fig. 1 are not representative) and are therefore subject to a wider range of weather conditions. Both these factors help to explain why in these cereals there is less seasonal fluctuation in the percentage of ergot-contaminated samples.

The occurrence of a 10- or 11-year weather cycle is well established and it might be expected that this variation in weather would affect the incidence of some plant diseases. However, there appears to be no published record of such an effect, and this survey of the percentage of ergot-contaminated cereal samples seems to be the first record of such a cycle in the incidence of a disease.

Although distinct rye varieties are now available there is no indication of their differing susceptibility to ergot. In wheat, however, there was some evidence of differences, spring varieties tending to be more frequently contaminated than winter varieties (Table 1). The date of sowing influences the time of flowering and in general the anthesis of winter-sown cereals is about 2 weeks earlier than that of spring-sown cereals and therefore will be subject to different weather conditions during the infection period. This difference in date of anthesis rather than any intrinsic susceptibility of the spring varieties is probably the cause of high ergot

contamination in the seasons studied. It was not possible to associate these results with any particular weather conditions which required more detailed meteorological data.

Differences in the susceptibility of wheat varieties to ergot infection during winter-sown variety trials in Hertfordshire were suggested by Willis (1953); Cappelle (?Desprez) appeared to contain more ergots than the other varieties listed. In the present survey no big difference was found between this and the other varieties which were studied.

Both ergot and loose smut (*Ustilago tritici* (Pers.) Rostr.) infect wheat at anthesis and therefore similar reactions of varieties to infection might be expected. However, most wheat varieties are embryo-susceptible to loose smut although they may later show field resistance (Batts & Jeater, 1958). It was not possible therefore to draw any comparison between varietal susceptibility to loose smut and to ergot.

The incidence of ergot-contaminated barley samples has been increasing in recent years (1950–7, Fig. 1) and it was thought that this might be due to the introduction of the more open-flowered Scandinavian-bred varieties, e.g. Carlsberg and Rika, and that a varietal differentiation similar to that found for loose smut (*Ustilago nuda* (Jens.) Rostr.) might be apparent (Marshall, 1959). However, in 1956 and 1957 there was no evidence to support this view (Table 2) and English-bred and Scandinavian-bred varieties were equally heavily contaminated with ergots.

While the proportion of barley and perhaps also of wheat samples which are contaminated appears to be increasing, there has been, since the peak years of 1927–8, an overall decrease in the incidence of rye ergots. It might have been expected that since 1918–19 improved seed-cleaning methods alone would have considerably reduced the percentage of ergot contamination. Whole or broken ergots which are approximately the same size as whole grains are not removed during normal cleaning processes and although they can be separated from the pure seed by floating off in brine solution (Dillon Weston & Taylor, 1942) or made ineffective by deep ploughing, these practices are not general and therefore no special control is used beyond routine cleaning.

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The meteorological data in Fig. 1 are published by permission of the Director-General of the Meteorological Office.

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THE INCIDENCE OF CERTAIN SEED-BORNE DISEASES IN COMMERCIAL SEED SAMPLES

III. *SEPTORIA* LEAF SPOT, OR BLIGHT, OF CELERY

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Commercial samples of celery seed have been submitted to the Official Seed Testing Station, Cambridge, from 1926 to 1958, for tests to determine the percentage infection by *Septoria* leaf spot (*Septoria apii-graveolentis* and *S. apii*). The disease test and a method for estimating the viability of *Septoria* spores is described. The percentage of samples with less than 1 % infection increased from 6·1 % in 1926–31 to 46·2 % in 1953–8 and the percentage of heavily infected samples decreased. But celery samples submitted since 1949 for purity and germination only were more heavily infected (in 1953–8 only 26·3 % had less than 1 % infection). In 1957–8, 78·8 % of all celery seed samples tested showed evidence of infection with *Septoria* spp.

No effect of variety or season on the incidence of disease was found during the survey.

Six out of thirty samples were found to carry viable *Septoria* as estimated by germination of pycnidiospores.

The similarity between the percentage germination of healthy seeds and those bearing *Septoria* pycnidia confirmed the general belief that *Septoria* spp. do not affect germination.

Introduction

Septoria leaf spot, or blight, is now the most serious disease of celery in this country. It can cause severe reduction in yield, decrease in the market value of the product or even complete loss of the crop. The disease is produced by two fungi, *Septoria apii-graveolentis* Dorogin and *S. apii* Chester, but the former is the commoner and more destructive pathogen in the United Kingdom.

The pathogens are carried over from one season to the next by infected seeds and under wet conditions inoculum from a small percentage of infected seed can spread rapidly to produce a high proportion of diseased seedlings. Infected plant debris in the soil also acts as inoculum.

Since 1926 the Official Seed Testing Station for England and Wales, Cambridge, has tested celery seed samples at the request of the sender for the presence of *Septoria* leaf spot.

During the past few years celery seed samples submitted to the O.S.T.S. for routine purity and germination tests, with no special request for a disease test, have been used for survey tests of infection only and results have not been reported to the sender.

Before 1944 the annual incidence of the disease was published in the annual

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report of the O.S.T.S. (in the *J. nat. Inst. agric. Bot.*) and since then in the *Rep. nat. Inst. agric. Bot.* (Annual Report), but a review and assessment of these figures has not been made. An analysis of the results of disease tests for *Septoria* spp. on celery seed from 1926–58 is now presented. The viability of pycnidiospores of *Septoria* spp. was investigated in 1957–8 as a guide to the reliability of the percentage infection quoted. The germination of healthy seeds was compared with that of seeds infected with *Septoria* spp. and is also reported.

METHOD

Infected seeds bear pycnidia which can be detected by examination with a good hand lens: the pycnidia swell when wet and are then much easier to distinguish. The percentage infection of a sample was determined by selecting 400 seeds by a standard seed-testing method and placing them on damp filter-paper in Petri dishes for 2 days at room temperature. The whole surface of the seed was then examined under a binocular microscope (×25). The pycnidia of *Septoria* spp. are readily distinguishable from other pycnidia, including those of *Phoma* (*P. apiicola* causes foot rot of celery and is also seed-borne), and similar but sterile bodies of other fungi which may be present on celery seed. No attempt was made to separate the two species of *Septoria* and a seed was regarded as infected if a pycnidium of either species was found on it. The number of pycnidia per seed was not estimated and infection was reported as a percentage of the seeds examined which carried one or more pycnidia.

If no pycnidia were found on 400 seeds a larger sample (about $\frac{1}{16}$ oz.) was shaken with a little water and left for about 24 hr. Drops from the washings were examined under a high-power microscope for the presence of pycnidiospores. If any spores were found the proportion of infected seeds was regarded as less than 1 in 400 and recorded as 'a trace', otherwise the sample was classed as 'apparently free from *Septoria* spp.'.

No indication of the viability of the pathogen was obtained by either of these methods. Assessment of viability by the germination of infected seeds in soil is unsatisfactory because infected seeds do not always produce infected seedlings and a suitable greenhouse test has not yet been developed. Agar-plating methods also proved unsuitable because *Septoria* spp. grow very slowly. More success has been achieved by keeping infected seeds, removed from filter-paper after the routine examination for pycnidia, and placing either a whole seed or a single pycnidium in a hanging drop of tap water in daylight at room temperature. Pycnidiospores could germinate within 24 hr. but the percentage germination was generally low (up to 5%). Tests for the viability of *Septoria* spores were made in this way on some of the samples submitted during 1957–8 as a guide to the reliability of the percentage infection quoted in the reports.

TABLE 1. *The incidence of* Septoria *spp. on samples of celery seed submitted to the O.S.T.S.*

Percentage infection ...	Percentage of samples in each infection group: Nil	Trace and less than 1	1–5	6–10	11–20	Over 20	No. of samples	No. of persons submitting samples
Seed year	A. Samples submitted specifically for disease test							
1926–7	0	0	10·3	27·6	24·1	38·0	29	13
1927–8	5·1	0	25·7	20·5	33·3	15·4	39	11
1928–9	12·5	0	0	25·0	62·5	0	8	*
1929–30	25·0	0	25·0	33·3	16·7	0	12	*
1930–1	0	0	50·0	10·0	20·0	20·0	10	*
1931–2	3·3	0	26·1	11·9	17·4	41·3	92	13
1932–3	9·5	0	22·3	10·2	13·4	44·6	157	18
1933–4	14·3	0	11·9	14·3	11·9	47·6	42	8
1934–5	10·2	0	24·5	16·3	22·5	26·5	49	8
1935–6	7·5	0	45·0	15·0	10·0	22·5	40	6
1936–7	18·2	3·0	39·3	9·1	3·0	27·4	33	7
1937–8	21·9	0	36·6	9·8	12·2	19·5	41	8
1938–9	22·2	5·6	36·0	13·9	2·8	19·5	36	9
1939–40	12·1	0	39·4	9·1	18·2	21·2	33	7
1940–1	0	21·5	35·7	7·1	21·5	14·2	14	*
1941–2	10·0	0	45·0	25·0	10·0	10·0	20	*
1942–3	0	11·8	29·4	29·4	11·8	17·6	17	*
1943–4	8·6	5·7	28·6	17·1	22·8	17·2	35	8
1944–5	14·5	4·3	31·9	15·9	14·5	18·9	69	10
1945–6	17·3	2·7	22·6	10·7	12·0	34·7	75	15
1946–7	9·2	9·2	17·4	15·6	11·9	36·7	109	16
1947–8	10·6	2·6	34·5	14·0	8·6	29·7	151	29
1948–9	32·8	3·2	20·8	10·4	13·6	19·2	125	22
1949–50	40·3	11·9	12·7	9·0	13·4	12·7	134	24
1950–1	37·1	6·5	16·8	12·1	12·9	14·6	124	26
1951–2	40·2	15·5	22·7	5·2	4·1	12·3	97	16
1952–3	35·5	11·3	30·6	8·1	6·5	8·0	62	10
1953–4	26·1	15·2	30·5	15·2	8·7	4·3	46	11
1954–5	26·6	20·3	23·4	7·8	4·7	17·2	64	15
1955–6	24·1	17·2	34·5	6·9	5·2	12·1	58	9
1956–7	36·6	12·2	39·0	4·9	7·3	0	41	6
1957–8	35·0	20·0	30·0	10·0	0	5·0	40	*
	B. Samples submitted for routine purity and/or germination tests only. (Results for 1951–2 and 1952–3 not available)							
1949–50	15·4	2·9	11·4	6·9	18·8	44·6	175	?
1950–1	16·2	3·8	15·2	7·6	17·1	40·1	105	24
1953–4	32·2	8·3	15·5	9·5	14·3	20·2	84	24
1954–5	23·6	5·4	16·4	10·9	10·9	32·8	55	17
1955–6	6·9	5·6	19·4	9·7	18·1	40·3	72	19
1956–7	17·2	3·5	17·2	10·3	13·8	38·0	29	12
1957–8	8·9	13·3	15·6	8·9	20·0	33·3	45	16

* = less than five persons submitting samples.

Part of Table 1 is published in the *Report of the committee on transactions in seeds* (Anon., 1957).

Results of survey

The results of tests on the samples submitted specifically for disease examination show that during the last 32 years there has been an increase in the proportion of samples apparently free from *Septoria* spp. (Table 1 A). From 1926 to 1931 an average of 6·1 % of the samples contained nil or less than 1 % of infected seeds compared with an average of 46·2 % from 1953 to 1958. Until 1953 to 4 samples determined as free from infection after examination for pycnidia on filter-paper were not examined further by the washing method described above and therefore very low infection levels were included in the nil category. Since then the increase in the proportion of these samples has been masked. There was a corresponding decrease in the incidence of more heavily infected samples and, although the percentage of infected samples with more than 5 % *Septoria* varied, there has been an overall decrease since 1926–7. An average of 72·4 % of samples received from 1926 to 1931 contained over 5 % of seeds infected with *Septoria* compared with only 22·9 % from 1953–8.

Samples which were submitted for purity and/or germination tests without a request for *Septoria* examination have been studied only since 1949–50 and similar trends are therefore not apparent (Table 1 B) but the infection levels in these samples were much higher than in those submitted for disease tests in the same year and from 1953–8 only 26·3 % had less than 1 % infection while 56·8 % had over 5 % infection.

In 1957–8, 78·8 % of all celery samples were infected.

Results of tests for the viability of *Septoria* spores

In 1957–8 the viability of *Septoria* spores from thirty infected seed samples was examined. Pycnidia from six samples contained spores which germinated readily in a hanging drop of tap water, suggesting that at least 20 % of the infected samples in 1957–8 contained viable *Septoria*.

Effect of *Septoria* seed infection on the germination of celery seed

During disease tests on five celery seed samples submitted in 1956–7 *Septoria*-infected seeds were segregated from healthy ones and separately tested for germination. The results are recorded in Table 2 which shows that there was no difference in the percentage germination in the two groups.

Discussion

The results of the survey of celery seed samples submitted specifically for disease tests showed that the percentage of samples with nil or less than 1 % infection with *Septoria* spp. has increased since 1926 with a sudden increase in 1948–9. In 1947 the problem of celery leaf spot was discussed by a meeting of celery raisers and growers and representatives of N.A.A.S. which recommended that all seed sold to the trade or to the public should be accompanied by a certificate showing the degree of *Septoria* infection. From the meeting arose the 'clean seed–clean

TABLE 2. *Germination of healthy and* Septoria*-infected celery seeds*

	Seeds with *Septoria*		Seeds with no *Septoria*	
% *Septoria* in original sample	No. tested	Percentage germination	No. tested	Percentage germination
30	250	68	250	58
41	450	52	450	54
40	200	45	200	39
29	58	76	142	65
44	89	78	111	64
Total	1047	—	1153	—
Average	—	58	—	55

celery' campaign (Dillon Weston & Cronshey, 1947) which stressed the importance of clean seed in the production of healthy crops. The success of this campaign was reflected also in the sudden increase both in the total number of samples submitted for disease test (109 in 1946–7 to 151 in 1947–8), and in the number of different people who requested tests (16 to 29). At that time the control of disease depended mainly upon the production of disease-free seed by regular spraying of seed crops with Bordeaux mixture, but since the development of an effective seed treatment by hot water (Bant & Storey, 1952) an additional means of control has been introduced and much celery seed is now hot-water-treated. By reducing field infection this has undoubtedly contributed indirectly to the increase in the percentage of samples in the low infection groups. However, some samples which are reported to contain infected seeds may be treated before sowing and therefore the infection level indicated by the survey figures since 1952 may be overemphasized.

Few of the celery seed samples submitted to the O.S.T.S. are described by a variety name and therefore no indication of varietal differences in the incidence of the disease, similar to that which was obtained during an equivalent survey on the incidence of loose smut in seed barley (Marshall, 1959), was possible on these celery seed samples.

Seasonal variations in weather are known to influence the incidence and severity of this disease in the field but there is little evidence from Table 1 that there has been any associated change in the proportion of diseased samples submitted such as that described for ergot in cereals (Marshall, 1960). This may be due to the very limited number of samples examined, but the effect of the 'clean seed–clean celery' campaign has masked any effect of weather since 1947.

The results of the survey are of limited significance because the origin of samples and the proportion they represent of the total celery seed supplied in each year is unknown, but it seems probable that the major part of the seed intended for sowing purposes is covered by the survey. The results do show, however, that those samples submitted specifically for disease have more seed which is free from *Septoria* spp. and very much lower infection levels than those not submitted for disease tests (Table 1) and it may be concluded from this that the senders of these samples for disease tests are aware of the problem, exercise some control over the

incidence of the disease and over the years have reduced the general level of infection.

Krout (1921), in experiments with the hot-water treatment of celery seed, found that spores of *Septoria* from untreated seed were usually dead after 3 years and only 2–3% were viable after 2 years, while in 1-year-old seed the viability was only slightly higher. But Macmillan & Plunkett (1942) showed that spores taken from pycnidia on infected leaf blades and stalks germinated in a few hours. This suggests that *Septoria* spores from pycnidia on seeds may either be in a dormant state or have a much lower viability than those from fresh vegetative material. Since the percentage germination of spores from seeds can be expected to be low, large numbers of spores, from as many different pycnidia as possible, must be examined before a reliable estimate of viability can be made. During 1957–8 20% of the infected samples which were examined were shown to carry viable spores of *Septoria* as determined by the ability of spores to germinate in water. Although a positive result is conclusive evidence that spores are alive, a negative result does not necessarily mean that they are dead and incapable of causing infection under favourable conditions.

No difference was detected between the germination of healthy seeds and those carrying pycnidia of *Septoria* spp. from five samples when grown under ideal germination conditions. This supports the general belief that the presence of *Septoria* pycnidia is not associated with a reduction in the germination of celery seed.

Although the general importance of disease control is more widely appreciated today than it was in 1926–7 when these tests were first started, it is clear that there is still a substantial proportion (78·8% in 1957–8) of the celery seed, moving in trade in this country, which is infected with *Septoria* spp. and therefore requires effective treatment. Since even low seed infections are capable of causing rapid increase in the disease to epidemic proportions when conditions in the field are suitable, many of the samples submitted to the O.S.T.S. for tests, unless effectively treated, are potentially able to produce severe infection in celery crops grown for market produce or seed. The survey has demonstrated that seed is still an important source of infection.

For data before 1953 I am indebted to Mr C. C. Brett, who started the testing of celery seed in the Official Seed Testing Station in 1926 and made all the tests up to 1949, and Mr A. Rothwell, who continued the work until 1952.

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THE INCIDENCE OF CERTAIN SEED-BORNE DISEASES IN COMMERCIAL SEED SAMPLES

IV. BUNT OF WHEAT, *TILLETIA CARIES* (DC.) TUL. V. EARCOCKLES OF WHEAT, *ANGUINA TRITICI* (STEIN.) FILIPJEV.

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Samples of commercial wheat seed submitted to the Official Seed Testing Station, Cambridge, have been examined from 1918 to 1957 for the presence of bunt balls (*Tilletia caries* (DC.) Tul.) and earcockles (*Anguina tritici* (Stein.) Filipjev) and the incidence of these organisms is reported.

The highest proportion of contaminated samples was found in 1921 (33 % with bunt balls and 9 % with earcockles). Since then incidence has decreased rapidly and is now steady at less than 1 % with bunt balls and nil or less than 0·1 % with earcockles. Low bunt-contamination was confirmed by finding no bunt spores in 122 samples in 1957.

The survey illustrates the effective control and almost complete elimination of a seed-borne disease and pest in the United Kingdom.

No seasonal effect on the incidence of either pathogen was found nor was any varietal susceptibility to bunt detected.

Introduction

In recent years it has become increasingly rare to find samples contaminated with either bunt balls (*Tilletia caries* (DC.) Tul.) or earcockles (*Anguina tritici* (Stein.) Filipjev), although for several years after the 1914–18 war wheat crops with 20–50 % of bunted ears were not uncommon (Moore & Moore, 1950). The incidence of these 'naked-eye diseases' in wheat seed samples submitted to the Official Seed Testing Station for England and Wales, Cambridge, has been reported each year since 1922 in the Annual Report of the O.S.T.S. (published in *Rep. nat. Inst. agric. Bot.* (Annual Report) or the *J. nat. Inst. agric. Bot.*), but the last summary (1918–29) was given by Eastham (1931–4). A comprehensive review of the incidence of bunt balls and earcockles in seed wheat samples from 1918–57 has therefore been made and is now presented.

Bunt of wheat

Method

Bunt may be present in wheat seed samples either as whole or broken parts of bunt balls (which can be seen with the naked eye and are detected during routine purity analyses) or, when the balls are broken, as spores distributed over the surface of the seeds (detected by examination of the filtered washings from samples of about 400 seeds).

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Results of survey

Table 1 shows the incidence of bunt-contaminated samples based on the presence of macroscopic portions of bunt found during purity analyses. In 1921, 33% of all wheat samples contained bunt, but since then the percentage has decreased and from 1951 less than 1% have been contaminated.

TABLE 1. *The incidence of bunt balls and earcockles in wheat samples*

Harvest year	No. of samples examined	% of samples containing bunt	% of samples containing earcockles
1918	?	5·7	2·7
1919	?	6·5	2·1
1920	1,926	8·0	4·0
1921	2,312	33·0	9·0
1922	1,866	15·7	2·8
1923	1,830	16·0	3·2
1924	2,326	14·5	3·0
1925	2,340	11·9	2·7
1926	2,423	5·6	3·9
1927	2,685	7·3	3·7
1928	2,538	5·7	2·6
1929	1,782	4·8	2·8
1930	2,142	4·6	2·2
1931	2,741	6·2	2·7
1932	4,575	5·1	2·5
1933	4,465	3·9	2·5
1934	4,896	4·1	2·3
1935	5,991	2·4	1·6
1936	7,499	2·7	1·5
1937	8,101	2·0	0·8
1938	8,100	1·6	0·9
1939	11,100	1·7	0·7
1940	9,610	1·2	0·3
1941	12,323	2·1	0·5
1942	14,860	1·7	0·4
1943	13,670	2·1	0·2
1944	5,000	2·1	0·2
1945	5,000	1·6	0·1
1946	5,000	2·2	0·2
1947	10,000	1·8	0·1
1948	10,000	1·4	0·1
1949	10,000	1·1	0·01
1950	12,900	1·0	0·02
1951	10,000	0·9	0·01
1952	12,000	0·6	0·01
1953	11,830	0·6	0·02
1954	7,200	0·7	0
1955	14,350	0·2	0·01
1956	17,900	0·3	0·01
1957	9,170	0·2	0

In 1957, 122 samples were examined microscopically for bunt spores but not one was found to be contaminated.

Table 2 shows that the incidence of macroscopic bunt-contaminated samples is similar in the 3 years 1955, 1956 and 1957 for seventeen varieties.

TABLE 2. *The incidence of bunt balls in varieties of wheat, 1955, 1956 and 1957*

Harvest year ...	1955		1956		1957		1954–7
Variety	No. of samples examined	Percentage of bunt-contaminated samples	No. of samples examined	Percentage of bunt-contaminated samples	No. of samples examined	Percentage of bunt-contaminated samples	Average % of bunt-contaminated samples
Pilot	100	1·0	46	0	37	2·7	1·1
Bersee	387	0·5	155	0·6	72	0	0·5
Wilma	227	0·4	123	1·6	112	0	0·6
Hybrid 46	702	0·4	890	0·2	625	0·2	0·3
Atle	1702	0·2	1833	0·7	617	0·5	0·4
Heines VII	792	0·1	606	0·5	266	1·1	0·4
Koga II	879	0·1	2894	0·2	1855	0·1	0·1
Cappelle Desprez	2147	0·1	4619	0·2	2802	0·1	0·1
Minister	273	0	126	0·8	77	0	0·2
Atson	476	0	956	0·3	314	0	0·2
Svenno	11	0	425	0·2	714	0	0·1
Peko	2214	0	879	0·1	327	0	Trace
N. 59	149	0	114	0	121	1·7	0·5
Banco	183	0	485	0	262	0·4	0·1
Eclipse	118	0	65	0	28	0	0
Holdfast	196	0	34	0	10	0	0
Yga Blondeau	101	0	77	0	43	0	0

Earcockles of wheat

Method

Earcockles are clearly visible with the naked eye and any contaminated samples are detected during routine purity analyses.

Results of survey

The incidence of earcockles in wheat seed samples is given in Table 1 which shows that the percentage of contaminated samples was highest in 1921 (9%) after which it decreased; since 1937 it has been less than 1% and after 1949 less than 0·1%. In 1954 and 1957 no earcockles were found in 7200 and 9170 samples, respectively.

Discussion

After 1918, contamination of wheat with bunt and earcockles first fell, then rose quickly to a maximum of 33% bunt and 9% earcockles in 1921. It has been suggested that the high infection of wheat with bunt after the 1914–18 war was due to the shortage of good seed stocks (Moore & Moore, 1950) and the correspondingly high earcockle contamination may be attributable to the same cause.

No seasonal effect such as that shown for the incidence of ergots (*Claviceps purpurea* (Fr.) Tul.) in cereal samples (Marshall, 1960) was apparent for either bunt or earcockles and since 1921 there has been at first a rapid and then a slower, but steady, decrease in the percentage of contaminated samples.

A survey made in 1925 by Eastham (1922–7) showed that while 18·0% of the O.S.T.S. samples examined showed macroscopic bunt, only 18·0% were apparently free from bunt spores. Thus the earlier figures of the incidence of bunt balls must considerably underestimate the proportion of samples contaminated with bunt spores. However, in 1957 only 0·2% of the samples contained macroscopic portions of bunt balls and none of the 122 samples examined microscopically contained bunt spores. The reduction in contamination is therefore real.

There was no evidence of any varietal susceptibility to bunt (Table 2), which confirms that most of the common varieties in the United Kingdom are susceptible to one or other of the physiologic races of bunt.

Bunt is effectively controlled by superficial disinfection of the seed before sowing and since about 1930 the increased popularity and now widespread use of seed dressings, especially the organo-mercurials, for wheat have undoubtedly caused this reduction in the incidence of bunt-contaminated samples. The deficiency payment scheme for wheat requires that farmers claiming payment on seed of their own sowing must sell it to a merchant and have it dressed with a mercurial dressing.

Somerville (1919) after experiments with steeps for the control of earcockle concluded that there was no practical method which would eliminate contamination without seriously reducing germination. He therefore recommended that contaminated wheat should not be used for seed. However, although seed-borne

earcockles are also carried in soil and better rotation and general cleanliness may help to account for the decline, improved seed cleaning processes have probably played the biggest role in practically eliminating this pest.

The survey demonstrates the control and almost complete elimination of a seed-borne disease (bunt) and a seed-borne pest (earcockle) in the United Kingdom.

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THE DETERMINATION OF THE REACTION OF BARLEY VARIETIES TO LOOSE SMUT

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(With 3 Text-figures)

The inoculation of barley with loose smut (*Ustilago nuda*) using various techniques has given results which do not reflect the known behaviour of varieties under natural conditions. No consistent results have been obtained, but all the varieties thus inoculated could become infected. No variety therefore possessed true physiological resistance and in the field some must escape infection. The inoculation techniques obviously overcame this escape.

A method has been devised which, with the varieties used, has reflected their behaviour in the field. Drops of coloured water or spore suspension are allowed to fall from a height on to ears at anthesis. Grains on the ears of varieties which do not become infected in the field do not become coloured or infected (depending on the drops used), while grains of varieties susceptible in the field do become coloured or infected. This is due to the glumes of the former group of varieties remaining closed, and so preventing access of smut spores under natural conditions.

The inoculation of wheat varieties with loose smut (*Ustilago tritici*) gives consistent results year after year. Various inoculation methods indicate the degree of susceptibility; some varieties are very susceptible, others are immune, while others are intermediate between the two (Batts, 1955; Oort, 1940). The reaction of barley varieties to *U. nuda* has, however, proved difficult to determine. A number of inoculation methods were used by Batts at the National Institute of Agricultural Botany, Cambridge, over several years, and unsatisfactory results were obtained. He used varieties the reaction of which was known from field observations. Inoculated ears which apparently were at the same stage of development often gave widely different infection percentages the following year. In addition, varieties such as Plumage Archer and Spratt Archer, which did not become infected in the field under natural conditions, could become highly infected after inoculation. Obviously therefore, the reaction of a variety under commercial conditions could not be determined by the inoculation methods used. Many other workers have also reported difficulties (Moore, 1936; Middleton & Chapman, 1941; Ross, Semenuik, Taylor & Jenkins, 1949; Tapke, 1955).

All the varieties inoculated by Batts at Cambridge became infected, and obviously none possessed true physiological resistance. It would appear therefore that varieties such as Plumage Archer and Spratt Archer somehow escaped infection in the field. Self-pollination is the rule in cereals, and in wheat anthesis is an obvious stage of development and always occurs when the ear is well clear of its

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sheath. In barley, however, anthesis often takes place while the ear is still within its sheath, and even when it occurs after ear emergence, extrusion of the anthers is not always a clear stage of growth. The glumes may remain closed and this phenomenon is largely a varietal character. A few workers have suggested that some barley varieties may escape loose smut infection by virtue of their closed glumes (Appel, 1915; Fromme, 1920). Tapke (1929) found, however, that the extent and duration of glume opening was practically the same for resistant and susceptible varieties. During a discussion on this subject Prof. P. H. Gregory suggested that it would be interesting to observe the effect of allowing water droplets to fall on to ears of a number of varieties, as this may provide an indication of the 'accessibility' of the ovary.

The problem of the determination of the reaction of barley varieties to loose smut was therefore investigated.

Materials and methods

In the spring of 1957 the following barley varieties were sown in field plots: Maythorpe, Plumage Archer, Spratt Archer and Earl (resistant to loose smut infection in the field), Proctor (semi-resistant), Piroline, Rika, Carlsberg, Kenia, Herta and Freja (susceptible). Some varieties were also sown in pots.

The ears were inoculated in June at pre-anthesis, anthesis and post-anthesis. To determine the stage of growth, florets from about the middle of the spike were examined before inoculation. To ensure inoculation at the correct stage of anthesis, the sheaths were carefully pulled back where necessary to allow access to the ear.

The following methods were used:

(1) Partial vacuum, 240 ears inoculated (Oort, 1939).

(2) Hypodermic needle, 440 ears inoculated (Poehlman, 1945).

(3) Gun spray. Spore suspension was sprayed on the spike from an aerograph gun. 110 ears inoculated.

Inoculum was prepared from fresh material obtained from the variety Fresia grown in the field. For each batch of inoculum two smutted ears were selected, cut into small pieces, placed in a small square of cheese cloth and squeezed through it into a beaker containing 500 c.c. of tap water. By this method a clear spore suspension free from extraneous plant material could be prepared. Fresh inoculum was used each day.

The inoculated ears were collected at maturity (15 August 1957) and kept separately in small packets for further experiments. The seeds of each ear were divided into two lots. One was examined for the presence of mycelium in the embryo and the other was sown in the field on 22 March 1958. Counts of smutted plants were made in the following August.

The preparation of the embryos was similar to that described by Simmonds (Batts & Jeater, 1958).

For the water droplet experiments, a tower made from aluminium alloy angle was used in the field (Gregory, Guthrie & Bunce, 1959). The tower consisted essentially of a frame supporting a large vertical asbestos tube 2·16 m. long and 10 cm. in

diameter, the lower end being placed 15 cm. above the ears of barley. A plastic shield with a hole in the centre 2 cm. in diameter was fixed to the frame below the asbestos tube, just touching the tips of the awns.

The frame was placed between rows in the barley crop and drops 3 mm. diameter were liberated from a specially prepared pipette at the top of the tube on to barley flowers in the field.

To hit the target accurately the falling drops had to be protected from draught: the asbestos tube gave this protection. In addition, drops were also liberated in the laboratory from a height of 5·3 m. on the ears of barley in pots.

The following drops were used: coloured drops (malachite green dissolved in water), plain water drops, and a spore suspension.

Barley plants grown in pots were taken to Rothamsted Experimental Station and loose smut spores were blown on to flowering ears of barley by placing the ears in a wind tunnel (Gregory, 1951). The following day water was dropped on to some of these ears in the laboratory from a height of 5·3 m.

At maturity, the grains were collected and examined by the embryo test method for the presence of loose smut.

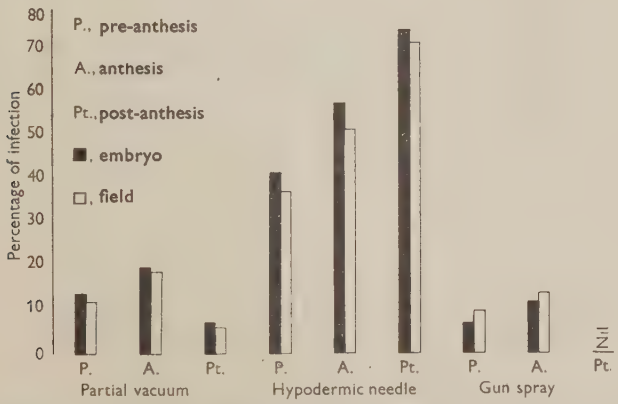

Fig. 1. Percentage infection (average of all varieties) after inoculation by various methods at different stages of development.

Experiments and results

Experiment 1. Comparison of inoculation methods at different stages of growth, and correlation between embryo and field tests

Averaging the results for all varieties, the hypodermic needle method gave the highest infection (Fig. 1). Maximum infection obtained in a single ear was 100% with hypodermic needle, 37% with partial vacuum, and 25% with gun spray. The optimum stage of growth varied to some extent with inoculation method, but least infection was obtained with the three methods at pre-anthesis. The percentage

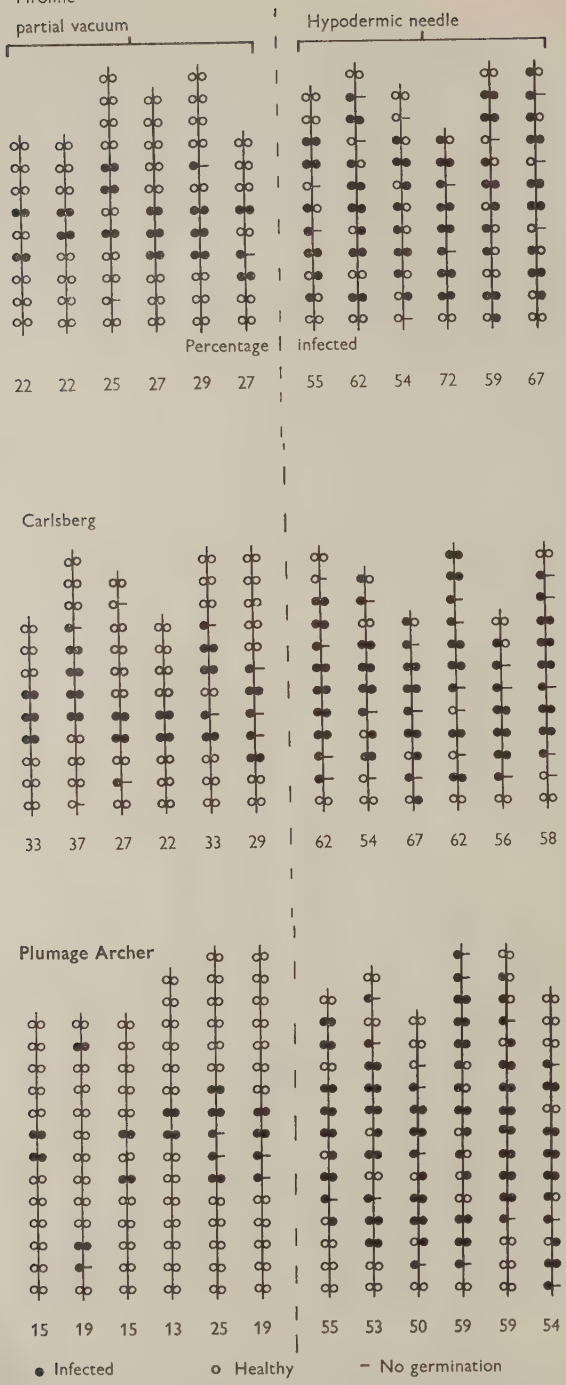
partial vacuum
Hypodermic needle
Percentage infected
22 22 25 27 29 27 55 62 54 72 59 67
Carlsberg
33 37 27 22 33 29 62 54 67 62 56 58
Plumage Archer
15 19 15 13 25 19 55 53 50 59 59 54
● Infected
o Healthy
– No germination

Fig. 2. The position of infected florets on the ears of three varieties, as determined by embryo and field tests after two methods of inoculation. Left side of ear embryo test, right side field test. ●, Infected; ○, healthy; —, no germination.

infection as determined by embryo and field tests was almost identical, the slight differences being well within the range infected from inoculation experiments.

All varieties became infected after inoculation. For example, after inoculation by hypodermic needle, the infection obtained in each variety was between 55 and 68%. While the results are presented as an average of all varieties, results obtained for each individual variety were examined but no consistently different pattern was found for any variety.

Only one partially infected ear was seen, but there was occasional infection on flag leaves and sheaths.

Experiment 2. *The position of infected florets in the ear*

Piroline, Carlsberg and Plumage Archer were inoculated at anthesis in the field by the partial vacuum and hypodermic needle methods. The inoculated ears were collected at maturity and were examined to determine the distribution of infection in the ear. From each ear the grains on one side were sown in the field and those on the other were used for embryo tests. The grains were sown in the order in which they were arranged on the rachis and the grains on the other side were likewise examined for infection.

In general, opposite florets were infected (Fig. 2) and the florets in the middle of the rachis were most often infected. This latter feature, however, was not quite so pronounced in the florets inoculated by the hypodermic needle method, where the infection did not follow such a set pattern. The percentage infection was slightly higher in Piroline and Carlsberg than in Plumage Archer.

Experiment 3. *The effect of inoculation on the viability of the seed*

Maythorpe, Plumage Archer, Spratt Archer, Proctor, Carlsberg and Herta were inoculated at anthesis by the hypodermic needle method. The ears were collected at maturity and a known number of grains from each ear were sown separately in each row the following spring. As the crop neared maturity, counts were made of the numbers of plants present and of the numbers of smutted plants.

There was no relationship found between the percentage infection and the percentage stand of the plants as shown in Table 1. This Table gives a further example of how varieties become similarly infected after artificial inoculation.

Water droplet experiments

A water drop falling freely in still air eventually reaches a maximum or terminal velocity which varies according to the size of the drop. Laws (1941) measured the terminal velocity of falling drops of different sizes and determined the distance required for these to reach 95% of this terminal velocity. He found that the 100% value was difficult to determine accurately.

Ideally, drops falling at terminal velocity should be used in all experiments on the effect of impact. As the maximum height for dropping was 5·3 m. in the

Table 1. *Percentage infection and percentage stand in ear rows of barley varieties inoculated with loose smut at anthesis with the hypodermic needle method*

Maythorpe		Plumage Archer		Spratt Archer		Proctor				Carlsberg		Herta	
% infection	% stand	% infection	% stand	% infection	% stand	% infection	% stand	% infection	% stand	% infection	% stand	% infection	% stand
100	50	100	100	100	25	100	90	82	100	100	80	100	60
91	100	100	78	82	74	100	80	80	91	100	46	100	56
90	92	84	40	75	89	100	50	80	50	100	44	100	44
80	100	70	100	75	31	100	42	80	50	100	36	100	27
77	100	69	93	62	100	100	32	80	75	100	28	92	100
69	75	67	100	60	100	100	27	78	68	100	27	91	91
55	75	62	73	54	79	100	25	75	100	88	80	80	72
50	89	55	100	33	60	100	20	75	73	86	70	75	53
50	65	50	75	20	48	96	90	67	93	85	100	60	83
42	70	50	55			92	100	64	100	80	45	57	58
40	82	42	100			92	100	63	80	75	75	50	53
40	91	0	91			89	100	62	81	75	72	45	75
33	100					89	90	56	56	75	67	25	73
33	100					86	100	55	100	75	66	20	62
26	100					86	87	46	69	72	63		
20	100					86	70	25	100	67	60		
						83	92			56	60		
										50	82		

laboratory and about 3 m. in the field, drops falling with maximum velocity were not available for these experiments and drops falling at less than their terminal velocity were used.

TABLE 2. *Distance required for a falling drop to reach* 95% *of its terminal velocity (from Laws,* 1941)

Diameter of drop (mm.)	Distance required for 95% T.V. (m.)
1	2·2
2	5·0
3	7·2
4	7·8
5	7·6
6	7·2

Experiment 4. *Effect of dropping coloured water on to barley ears*

Drops of coloured water measuring 3 mm. in diameter were liberated on to ears of Earl, Spratt Archer, Plumage Archer, Proctor, Carlsberg and Herta in anthesis. From five to eight drops were allowed to fall on each ear and six ears of each variety were thus treated. The ears were collected and each floret was examined for the presence of colour on the style or ovary.

The lemma and palea of all varieties were coloured. In Earl there were no coloured ovaries or styles and only one in Spratt Archer and Plumage Archer (Fig. 3). In Proctor, Carlsberg and Herta, however, out of 184, 194 and 184 ovaries examined, respectively, 9, 27 and 33% were coloured.

Experiment 5. *The effect of dropping: (a) spore suspension; (b) dusting spores and dropping water; and (c) dusting spores, on to barley varieties*

Plumage Archer, Spratt Archer, Maythorpe, Proctor, Carlsberg, Piroline and Herta were used in this experiment. From five to eight drops of loose smut spore suspension were made to fall on the ears in anthesis in the field, six ears of each variety being treated in this way.

Another set of twelve ears of each of these varieties were dusted with loose smut spores with a small brush at anthesis and on six of these dusted ears of each variety, five to eight drops of water were allowed to fall and the remaining six were left dry. The ears were labelled and at maturity the grain was collected and examined for the presence of loose smut infection by the embryo test method.

Carlsberg, Herta, Piroline and Proctor became infected after all treatments, with Proctor showing the least infection (Table 3). The association of water with the spores virtually doubled the infection produced by applying dry spores only. Plumage Archer and Spratt Archer remained uninfected, while Maythorpe became slightly infected after one treatment. The results of this experiment closely reflected the results of Exp. 4 (Fig. 1), the varieties with most colour on the ovaries also having the greatest amount of infection.

Experiment 6. *The effect of blowing loose smut spores on to barley ears in the wind tunnel, followed by dropping water on some ears*

Spores of loose smut of barley were blown in the wind tunnel on to the ears of Plumage Archer, Spratt Archer, Proctor and Carlsberg in anthesis. One set of ears

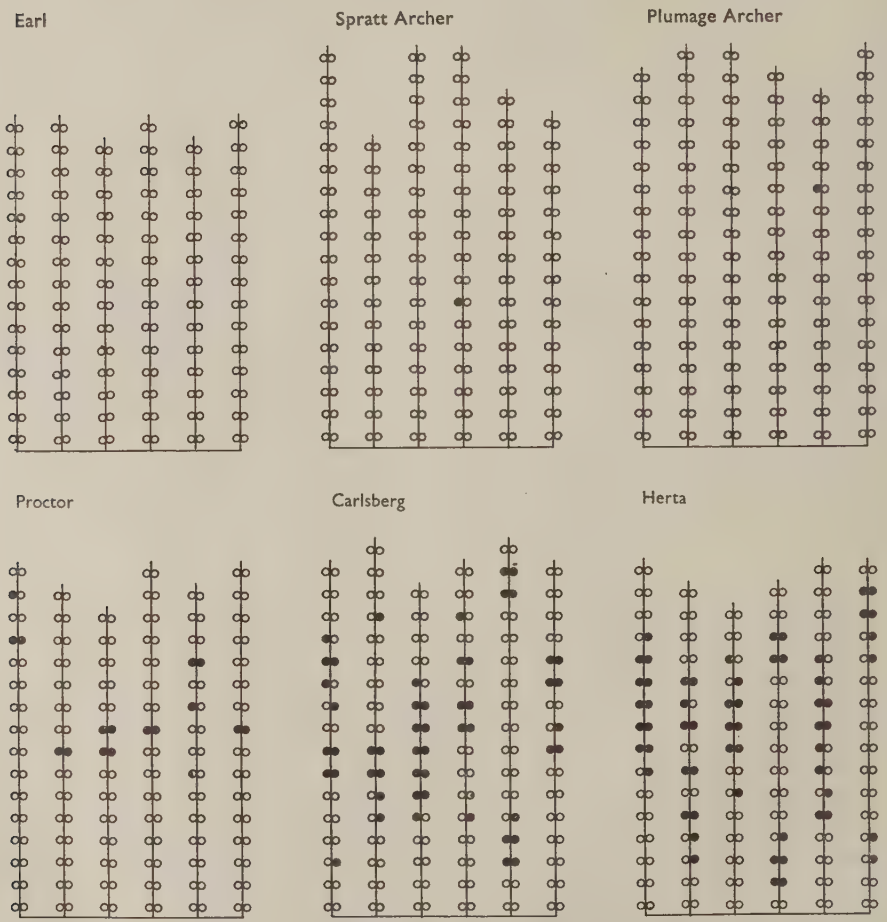

Fig. 3. The position of coloured ovaries in six ears of six varieties after dropping coloured water. ●, ovaries coloured by dye; ○, ovaries not coloured.

was impacted for 5 min. employing a wind speed of 1·8 m./sec. and another set at 3·6 m./sec. for 5 min. The liberation of loose smut spores was according to the description of Gregory (1951). Six ears of each variety were used in the experiment. On three of them from five to eight drops of water were dropped from a height of

5·3 m. in the laboratory. At maturity the grains were collected and examined by the embryo test method for the presence of loose smut infection.

The ears were covered with the spores when treated in the wind tunnel for 5 min. at wind speeds of 3·6 and 1·8 m./sec., respectively, and the infection was nearly

TABLE 3. *The effect of dropping spore suspension and of dusting spores with and without water droplets on to ears of barley varieties at anthesis*

Variety	Treatment	No. of grains examined	No. of embryos infected
Plumage Archer	Dropped spore suspension	140	0
	Dusted spores and dropped water	137	0
	Dusted spores only	136	0
Spratt Archer	Dropped spore suspension	145	0
	Dusted spores and dropped water	138	0
	Dusted spores only	141	0
Maythorpe	Dropped spore suspension	106	2
	Dusted spores and dropped water	125	0
	Dusted spores only	123	0
Proctor	Dropped spore suspension	113	2
	Dusted spores and dropped water	116	5
	Dusted spores only	113	1
Carlsberg	Dropped spore suspension	132	12
	Dusted spores and dropped water	130	14
	Dusted spores only	130	7
Piroline	Dropped spore suspension	131	14
	Dusted spores and dropped water	129	10
	Dusted spores only	132	4
Herta	Dropped spore suspension	146	16
	Dusted spores and dropped water	138	12
	Dusted spores only	139	8

TABLE 4. *The reaction of barley ears after exposure to loose smut spores in a wind tunnel for 5 min., followed by water drops*

Variety	Treatment	Wind speed (m./sec.)	No. of grains examined	No. of embryos infected
Plumage Archer	Blown spores only	3·6	63	0
		1·8	63	0
	Blown spores and dropped water	3·6	65	0
		1·8	62	0
Spratt Archer	Blown spores only	3·6	69	0
		1·8	59	0
	Blown spores and dropped water	3·6	73	0
		1·8	70	0
Proctor	Blown spores only	3·6	47	2
		1·8	57	3
	Blown spores and dropped water	3·6	60	5
		1·8	62	5
Carlsberg	Blown spores only	3·6	57	4
		1·8	45	3
	Blown spores and dropped water	3·6	54	7
		1·8	56	8

the same in Carlsberg and Proctor at different speeds. The amount of infection increased slightly when water was dropped on the blown ears. No infection was found in Plumage Archer and Spratt Archer (Table 4).

Discussion

Inoculation with the hypodermic needle gave the highest percentage of infection. Poehlman (1945) and Cherewick & Cunningham (1956) obtained a similar result. By this method, inoculum is introduced into each floret, but after inoculation with the partial vacuum and gun spray methods, many florets escape infection. This is somewhat surprising because it might be expected that under partial vacuum, the glumes would be drawn apart and so allow entry of inoculum. The stage of development of the ovary was not particularly critical, some infection being obtained over all stages of anthesis, some grains even 6 mm. long being susceptible. A similar range has also been found by others (Shands & Schaller, 1946; Cherewick & Cunningham, 1956).

Wells & Platt (1949) observed that inoculation of barley with loose smut resulted in lower viability of the seed, the reduction depending to some extent on variety. Russell (1950) and Thren (1938) also reported injury to the barley embryo after artificial inoculation with *U. nuda*, but no consistent result has been obtained in the numerous counts made to check this phenomenon. In naturally infected seed, however, Russell (1950) found no evidence of injury due to infection since embryo examination gave a very good indication of the amount of loose smut which could be expected in the subsequent crop. In the present experiments, a very close correlation was found between embryo tests and field infections in all inoculations.

As previously indicated, all varieties may become infected after inoculation, the results in no way reflecting the behaviour of the variety in the field under natural conditions. The results of the water droplet experiments, however, approximated very closely to the reaction of the varieties to loose smut in the field. When coloured water was used, almost a third of the ovaries of Carlsberg and Herta ears were dyed, less than a tenth of the ovaries of Proctor, while there was only one ovary dyed in the ear of Spratt Archer and Plumage Archer, and none in Earl. The experiments with spore suspension and spores with and without water droplets also closely reflected the behaviour of the varieties in the field.

When spores were blown over the ears of different varieties, a method simulating natural conditions, Spratt Archer and Plumage Archer did not become infected, while Carlsberg and Proctor did, the former more than the latter. When water droplets were allowed to fall on the ears after the spores had blown over them, infection was increased by about 50% in Carlsberg and Proctor but Spratt Archer and Plumage Archer remained healthy. It would appear that the water droplets had penetrated between the glumes of the varieties which became infected, carrying the spores in. It has been found that loose smut infection is greatest in wet seasons (Tapke, 1931; Batts, 1956). One reason for this is that pollination is delayed during wet dull weather, the florets remaining open, while most rapid pollination takes

place during dry bright weather when the cereal flowers are open for only a comparatively short period. Another reas on maywell be that, as found by Gregory (1952), *Ustilago* spores are brought down by raindrops and some of the spores could easily be carried to a position around the ovary within the glume.

In the determination of the reaction of barley varieties to loose smut, it appears that the results obtained from artificial inoculation techniques would detect those varieties which have true physiological resistance. In a search for material for breeding for resistance, true physiological resistance is obviously most valuable, and inoculation techniques may profitably be used. For determining the behaviour of varieties in the field, however, such techniques are of no value. The experiments described have shown that while all the varieties tested are susceptible to *U. nuda*, those which do not become infected in the field under natural conditions escape infection due to the inaccessibility of the ovary It is well known that barley varieties vary in the number of closed flowers they possess, and it is varieties which possess closed flowers which escape infection in the field.

The dropping of coloured water droplets on to barley ears is obviously a technique for determining whether or not the spikelets of a given variety open for pollination. The number of ovaries which become coloured is a direct indication of the number of spikelets which open. Varieties in which a number of ovaries become coloured would be susceptible to loose smut in the field, while those where no ovaries became coloured would escape infection in the field, i.e. would be field resistant. Thus of the varieties used in the water dropping experiments, Carlsberg and Herta would be described as susceptible in the field, Proctor fairly resistant and Earl, Spratt Archer and Plumage Archer would be virtually field resistant. Such a description is in complete agreement with the known behaviour of the varieties in the field under natural conditions.

Our thanks are due to Rothamsted Experimental Station for permission to use the wind tunnel.

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THE COLIFORM BACTERIA ASSOCIATED WITH POTATO BLACK-LEG AND OTHER SOFT ROTS

I. THEIR PATHOGENICITY IN RELATION TO TEMPERATURE

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(With Plate 2)

Twelve glasshouse trials using twenty-five isolates of coliform soft-rot bacteria obtained from various countries showed that all the isolates produced typical black-leg of potato at temperatures of 76° F. (24·5° C.) or over, while some of them could also produce the disease at temperatures below 66° F. (19° C.).

Thus the isolates fall into two main groups of which the high-temperature group originates mostly from tropical or subtropical countries or from plants grown in heated glasshouses, whereas the low-temperature group is indigenous in the north temperate regions. The high-temperature group comprises *Pectobacterium carotovorum* (*Erwinia carotovora*), *P. carotovorum* var. *chrysanthemi* (*E. chrysanthemi*) and *P. carotovorum* var. *aroideae* (*E. aroideae*). The low-temperature group consists of one variety, *P. carotovorum* var. *atrosepticum* (*E. atroseptica*).

This temperature relation provides a possible means of discovering the origin of the black-leg pathogen in crops grown from Scottish seed in certain tropical or subtropical countries.

As a result of this investigation it is considered that all soft-rot coliform bacteria are varieties of a single species, *P. carotovorum* (Jones) Waldee.

Introduction

The problem of interrelations within the complex group of organisms known as the 'soft-rot coliform bacteria' has existed for more than half a century. Ever since Jones (1901) described *Bacillus carotovorus* as the cause of soft rot of carrot, van Hall (1902) *B. atrosepticus* as the cause of potato black-leg and Townsend (1904) *B. aroideae* as the cause of soft rot of arum lily, workers have disputed whether these three should be designated as different species, or variants of a single species. In 1920, Erwin Smith stated that *B. phytophthorus* (the name given to *B. atrosepticus* by Appel (1902)) was 'not sufficiently distinguished from *B. carotovorum* Jones'. Notwithstanding the protracted study of these organisms, the position still remains essentially the same.

The nomenclature of the soft-rot bacteria has become confused because several generic names have been proposed for them. American workers adopted the genus *Erwinia* Winslow *et al.*, and when the genus *Bacillus* Cohn was made invalid for rod-shaped non-sporing organisms, British workers placed the plant pathogenic

coliforms in the genus *Bacterium* Ehrenberg. However, *Bacterium* was rejected by international agreement in 1953, and this decision forced British bacteriologists to use a new name. Some immediately accepted *Erwinia*, but Dowson suggested the adoption of the *genus* Pectobacterium (Waldee, 1945) for the soft-rot organisms, leaving *Erwinia* for the coliforms causing dry necroses, galls or wilts. Although this suggestion has not yet been generally accepted, we propose to use the name *Pectobacterium* for the organisms discussed in this and the following paper.

The basis for separating the soft-rot coliform bacteria into species, variants and *forma speciales* has always been controversial. It has rested largely on characteristics of fermentative ability and pathogenicity, while little attention has been paid to serology or the newer tests now widely employed by medical bacteriologists, such as growth in cyanide media and production of amino-acid decarboxylases. Much of the work on pathogenicity has been unsatisfactory, most of the tests having been made by inoculating soft, fleshy plant organs such as potato tubers, cucumber fruits and onions. Amongst growing plants the potato has been most often used because of its commercial significance and the fact that potato black-leg is probably the most important bacterial soft-rot disease. Some of the earlier investigators found that certain isolates when inoculated into potato stems readily produced black-leg disease, while other isolates did not; and it was noted that those producing black-leg had almost invariably been isolated from potato, while the remainder usually came from other hosts. Coupled with this were certain biochemical differences; and some workers considered that the etiological agent of black-leg disease should be separated from the other soft-rot coliforms and called *Erwinia atroseptica* (van Hall) Jennison (*P. carotovorum* (Jones) Waldee var. *atrosepticum* Dowson). The most recent exponents of this view are Burkholder & Smith (1949), who maintained there were two distinct species, *E. atroseptica* and *E. carotovora*. At that time they did not recognize the existence of *E. aroideae*, generally regarded as an organism which produces acid but no gas from carbohydrates. Later, however, Holdeman & Burkholder (1956) claimed to have isolated 'true' anaerogenic *E. aroideae* from tobacco.

In recent years other investigators, notably Jones (1950) and Hellmers & Dowson (1953), claimed it was possible to produce black-leg of potato with all soft-rot coliform isolates. Hellmers & Dowson considered that inconsistency in the results of pathogenicity tests could be explained through their discovery that the bacteria must be introduced directly into the vascular bundles to obtain infection. Using this method, they were able to cause black-leg with an American isolate which was said by Burkholder & Smith to be non-pathogenic towards potato stems.

Because published work proved so contradictory, experiments on potato black-leg were started at Scientific Services in 1951. Black-leg is important in connexion with the Certification Scheme and the seed potato export trade, and the work reported here is part of a re-examination of all aspects of the disease being carried out at Edinburgh.

Pathogenicity tests on potato plants

Early experiments at Edinburgh tended to confirm the view of Burkholder & Smith that at least two species were involved, and some isolates were found to be unable to produce black-leg even when introduced directly into the vascular tissue of the stem (Noble & Marshall, 1952). In 1955–6 work was continued by D. C. Graham using a much greater range of isolates, and employing Hellmers & Dowson's technique. Again the results were inconsistent, so joint work was arranged to discover the reasons for these discrepancies. We carried out two tests together at Cambridge in April and May 1957 and then a series of ten further experiments independently in Cambridge and Edinburgh throughout 1957 and 1958.

Materials and methods

During the twelve trials, twenty-five isolates were employed, but not all were used in every test. The source and identity of each isolate is given in Table 1. Cultures of particular interest include Holdeman & Burkholder's 'true *aroideae*' (strain H2); the organisms causing slow wilt of carnation (Lelliott, 1956) (strain 402), bacterial blight of chrysanthemum (Burkholder, McFadden & Dimock, 1953) (strain 399), and bacterial disease of guayule (Starr, 1947) (strain 398); and also the organism recently found to be the cause of soft rot of pineapple in Malaya (Johnson, 1957) (strain 1172). Strain 377 is also of interest, as we consider it to be identical with the organism described by Sabet (1954) as *E. carotovora* f.sp. *zeae*, the cause of bacterial rot of maize in Egypt. Strain EP3 is the organism causing leaf rot of *Philodendron* described by Miller (1956) from Florida. Cultures were grown on meat infusion agar slopes for 24 hr. at 27° C. and the growth emulsified in 5 ml. water for inoculation.

Potato plants were grown in 6 in. pots of sterile soil in cool glasshouses, using the variety Sharpes Express at Cambridge and Majestic at Edinburgh. Both varieties are known to be susceptible to black-leg from field observations.

Inoculation wounds were made with sterile safety-razor or scalpel blades as described by Hellmers & Dowson (1953), by cutting across the wings on the stems, going deep enough to sever one of the main vascular bundles. The bacterial suspension was inserted into the wounds with sterile glass pipettes, and the wounds bound with 1 in. wide self-adhesive tape. Controls were inoculated with sterile water. Each stem was usually inoculated in two places, one at the base about 1 in. above soil level, the other 3–4 in. below the growing point. In each experiment all the stems of between four and ten plants were inoculated with each isolate, with three plants as checks. At Cambridge the plants were covered with large bell jars and kept in the glasshouse, but at Edinburgh, plants were maintained in small separately heated and ventilated cubicles in the glasshouse. Humidity was kept near 100% by frequent damping down of the walls and floor. The maximum and minimum air temperatures were recorded daily so that the average temperatures could be calculated for the duration of the tests, which usually lasted about 7 days.

TABLE 1. *Isolates used in black-leg inoculations*

Ref. no.	Plant	Source	Date of isolation	Name of organism as received	Name of organism considered to be correct
SR2/1	Potato stem	S. Rhodesia	1955	—	*P. carotovorum* var. *atrosepticum*
HT/1	Potato stem	Scotland	1955	—	*P. carotovorum* var. *atrosepticum*
QB4	Potato stem	Scotland	1955	—	*P. carotovorum* var. *atrosepticum*
123	Potato	U.S.A.	?	*E. aroideae*	*P. carotovorum* var. *aroideae*
14B	Potato tuber	England	1954	*E. carotovora*	*P. carotovorum*
340	Avocado pear	Israel	1954	*E. carotovora*	*P. carotovorum*
312	Potato tuber	Denmark	1952	*E. carotovora*	*P. carotovorum*
377	Maize	S. Rhodesia	1956	*E. carotovora*	*P. carotovorum*
H2	Tobacco	U.S.A.	1955	*E. aroideae*	*P. carotovorum* var. *aroideae*
EP3	Philodendron	U.S.A.	1955	*E. chrysanthemi* var. *philodendri*	*P. carotovorum* var. *chrysanthemi*
370	Calla Lily	Denmark	1955	*E. aroideae*	*P. carotovorum* var. *aroideae*
A81V	Avocado pear	Israel	1954	*E. aroideae*	*P. carotovorum* var. *aroideae*
398	Parthenium	U.S.A.	1946	*E. carotovora* f.sp. *parthenii*	*P. carotovorum* var. *chrysanthemi*
399	Chrysanthemum	U.S.A.	1955	*E. chrysanthemi*	*P. carotovorum* var. *chrysanthemi*
402	Carnation	Denmark	1955	*E. carotovora* f.sp. *dianthicola*	*P. carotovorum* var. *chrysanthemi*
39	Potato stem	Scotland	1957	—	*P. carotovorum* var. *atrosepticum*
H25	Potato tuber	Denmark	1952	*E. atroseptica*	*P. carotovorum*
H35	Potato tuber	Denmark	1952	*E. carotovora*	*P. carotovorum*
H904	Iris	U.S.A.	1946	*E. carotovora*	*P. carotovorum*
C1	Potato	Israel	1956	*E. atroseptica*	*P. carotovorum* var. *atrosepticum*
C5	Potato	Israel	1956	*E. atroseptica*	*P. carotovorum* var. *atrosepticum*
119V	Tomato fruit	Israel	1958	*E. aroideae*	*P. carotovorum* var. *aroideae*
B1	Potato stem	Scotland	1957	—	*P. carotovorum* var. *atrosepticum*
74V	Maize	Israel	1957	*E. aroideae*	*P. carotovorum* var. *aroideae*
1172	Pineapple	Malaya	1956	*E. carotovora*	*P. carotovorum*

Results of inoculations

In the first trial at Cambridge during the period 29 April 1957 to 5 May 1957, strains H25, H35, H904, 123, SR2/1, H2, EP3, and 377 were used and of these only SR2/1 and H904 gave a black-leg reaction; the former caused rapid rotting, wilting and collapse, but the latter was only weakly pathogenic with slow spread of the rot. Most of the strains which failed to produce the typical disease gave only small localized lesions at the site of inoculation. These were the results which Graham had predicted from previous experience of the isolates. We quickly realized that the plants had been kept in a cold glasshouse and we considered that temperature of incubation might have a more significant effect than was generally realized. Accordingly, a second experiment was carried out between 3 May 1957 and 9 May 1957 using similar plants, but these were transferred to a heated glasshouse after inoculation. The effect was most striking, all strains producing severe black-leg symptoms within 4 days. It thus appeared that temperature was the controlling factor in disease production, and the further ten tests, using an increased number of strains, amply confirmed our first impressions. Unfortunately, neither

TABLE 2. *Summary of results of temperature infection experiments*

	Temperature ranges					
	Lower		Higher			
Strain	60–65° F.	66–70° F.	71–75° F.	76–80° F.	81–85° F.	86–90° F.
SR2/1*	+ + +	+ + + +	+	+ + +	+ +	+ +
HT/1*	+ +	+ +	+	+	+ +	+
QB4*	+ +	+ +	+	+	+ +	+
123	− −	− + +	+	+	+	+
14B	− −	− −	−	.	+	+
340	−	−	−	+	+	+
312	−	+	−	.	.	+
377	− −	− −	−	− +	+	+ +
H2	−	− − −	−	− +	+ +	+
EP3	−	+ −	+	+ +	+	+
370	−	−	+	+	+	+
A81V	−	− + +	.	+	+	+
398	− −	− +	+	.	+	+
399	− −	+ +	+	.	+	+
402	− −	− −	+	.	+	+
39*	+ +	+ +	.	+	+	+
H25	.	− +	+	+	.	.
H35	.	− +	.	+	.	.
H904	.	+	.	+	.	.
C1*	+	+	.	+	+	+
C5*	+	+	.	+	+	.
119V	−	−	.	+	+	+
B1*	+	+	.	+	.	+
74V	−	−	.	+	.	+
1172	−	−	.	+ +	+	+

Each positive or negative sign represents the result of a test carried out within a given average temperature range. The asterisk marks those strains that infect at the lowest temperature, i.e. below 66° F. These correspond to *Pectobacterium carotovorum* var. *atrosepticum*.

+ve = black-leg production; −ve = no reaction; . = reaction not recorded.

of us had controlled-environment equipment at our disposal, but by adjusting the heating and shading, and the use of extractor fans in the glasshouse, we obtained considerable temperature ranges.

Table 2 summarizes the results of all experiments, but only the average temperatures throughout the duration of each experiment are given and these are grouped within arbitrary temperature ranges for ease of presentation. The reactions of the variety Majestic inoculated with a strain capable of causing black-leg at a high temperature only (strain 377) and kept at high and low temperatures are illustrated in Pl. 2.

Discussion

The discovery of this temperature-pathogenicity relation is important in regard to the controversy as to whether or not all soft-rot coliform bacteria are capable of causing black-leg of potato. Examination of the sources of isolates shows that most of the group of organisms causing black-leg only at the higher temperatures correspond to the *E. carotovora*, *E. chrysanthemi* and *E. aroideae* of American workers (our *P. carotovorum*, *P. carotovorum* var. *chrysanthemi* and *P. carotovorum* var. *aroideae*, respectively). These usually originated in the tropics or subtropics, or were found associated with such plants as calla lilies, which are grown in heated glasshouses in temperate climates. Such isolates, pathogenic only at higher temperatures, are obviously strains evolved under warmer conditions than are normally experienced in Britain. Similarly the group of isolates inducing black-leg at the low temperatures correspond to the American *E. atroseptica* (our *P. carotovorum* var. *atrosepticum*), and it is this organism which is the causal agent in the cool climate of Scotland. Since 1955, more than 200 isolations have been made from sixteen different black-leg-infected potato varieties, and the pathogenicity of many of the isolates has been checked. All belong to the low-temperature group, and as far as can be ascertained the Scottish black-leg organism is a perfectly homogeneous variant.

Furthermore, this temperature relation provides a possible means of discovering the origin of black-leg infection in crops grown from imported Scottish seed in certain tropical and subtropical countries. For instance, the organisms obtained from Israel and Southern Rhodesia, isolated from potatoes grown from Scottish seed, were found to belong to the low-temperature group; a result which additionally supports the view that the black-leg pathogen is carried with the tuber to the importing country, and that the disease there need not be caused by indigenous strains of soft-rot coliforms.

Finally, we consider that the results lend support to the view that all soft-rot coliform bacteria are variants of a single species, *P. carotovorum*.

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Fig. 2

Fig. 1

GRAHAM AND DOWSON—*Coliform bacteria associated with potato black-leg. I*

(*Facing p.* 56)

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EXPLANATION OF PLATE 2

Fig. 1. Potato plant (var. Majestic) inoculated with a high-temperature strain, and kept at a low temperature (average 62° F.). Photographed 7 days after inoculation. Note the localized lesion on the stem; there is no spread of the bacteria from this region.

Fig. 2. Potato plant (var. Majestic) inoculated with a high-temperature strain and kept at a high temperature (average 88° F.). Photographed 4 days after inoculation. Note the typical wilting of the plant and blackening of the stem base; the organisms have also spread down the petiole and into the leaf.

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THE COLIFORM BACTERIA ASSOCIATED WITH POTATO BLACK-LEG AND OTHER SOFT ROTS

II. BIOCHEMICAL CHARACTERISTICS OF LOW- AND HIGH-TEMPERATURE STRAINS

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The biochemical characteristics of twenty-five isolates of soft-rot coliform bacteria from different countries are recorded. Because of the variability of the reactions, strains capable of producing potato black-leg at both low and high temperatures, or at higher temperatures only, cannot be distinguished from one another with certainty by biochemical tests alone. They do, however, give an indication of potential pathogenic reactions. Thus the low-temperature strains correspond with *Pectobacterium carotovorum* var. *atrosepticum* and are distinguished from the high-temperature isolates by their gas production, rapid fermentation of lactose and maltose, methyl red and Voges–Proskauer reactions and failure to give a positive indole test.

Because of their delayed lactose fermentation the chrysanthemum blight pathogen, the carnation slow wilt organism, the guayule bacterium and the organism causing leaf rot of *Philodendron* are classified as a new variety of *P. carotovorum* (Jones) Waldee.

The diagnosis of the coliform soft-rot bacteria should be based on qualitative and quantitative biochemical reactions rather than on host range.

Introduction

In the preceding paper we pointed out that all isolates of soft-rot coliform bacteria were capable of causing black-leg in the glasshouse if the inoculated plants were incubated at appropriate temperatures, and that strains could be conveniently divided into low- and high-temperature groups. Those producing black-leg below an average temperature of 66° F. (19° C.) were considered to correspond to *Pectobacterium carotovorum* var. *atrosepticum*.

Biochemical tests were made on the isolates concurrently with the pathogenicity experiments. This was done, first, to confirm the identity of the organisms as soft-rot coliform bacteria; and secondly, to find whether any biochemical reaction or group of reactions would distinguish between the low- and high-temperature strains. For details of the organisms employed in the biochemical tests, the reader is referred to Table 1 in Graham & Dowson (1960).

Biochemical tests

Methods

Fermentation tests. Salicin, sucrose, glucose, lactose and maltose were incorporated at 1 % concentration in an inorganic (basal) medium, using ammonium phosphate as a nitrogen source, together with bromothymol blue as an indicator, at pH 7·2. Final readings were taken after incubation for 21 days at 26° C.

Liquefaction of gelatin. Liquefaction was observed in nutrient gelatin stab cultures incubated for 30 days at 22° C.

Reduction of nitrate. Nitrate reduction was determined by growing the organism in a medium containing 1 % peptone (Evans) and 0·1 % potassium nitrate for 48 hr. at 26° C. and testing for the presence of nitrite with the dimethyl-1-naphthylamine sulphanilic acid reagent.

Liquefaction of pectate gel. Stab cultures were made in Wieringa's pectate gel medium; details of preparation are given by Dowson (1957). Cultures were incubated at 26° C., and readings taken after 14 days.

Methyl red and Voges–Proskauer tests. Organisms were grown in ordinary glucose–phosphate–peptone (Evans) medium for 5 days at 26° C. The culture fluid was then divided into two, one portion being tested with methyl red indicator, the other for the presence of acetylmethylcarbinol by the caustic potash–creatine method.

Gluconate test. The medium employed consisted of potassium gluconate 40·0 g.; peptone (Evans) 2·5 g.; K_2HPO_4 1 g.; distilled water 1000 ml.; adjusted to pH 7·0; sterilized at 10 lb./sq.in. for 10 min. After incubation of the culture for 48 hr. at 26° C., an equal volume of Benedict's qualitative reagent for reducing sugars was added and the tubes placed in boiling water for 10 min. In this test a positive reaction is indicated by a heavy yellowish-brown precipitate of cuprous oxide.

Ethyl alcohol agar test. Cultures were grown on alcohol agar prepared according to the method of Massey (1924). They were incubated at 26° C., and final readings were taken after 21 days.

Indole production. Organisms were grown in 1 % Bacto–Tryptone broth for 48 hr.; the presence of indole was detected with Kovacs's *p*-dimethylaminobenzaldehyde reagent.

Hydrogen sulphide. A similar medium to that used for the indole test was employed. H_2S was detected by lead acetate papers suspended above the medium. Cultures were incubated for 21 days at 26° C. before final readings were taken.

Results

Table 1 summarizes the results of the tests made on the twenty-five isolates of soft-rot coliform bacteria. None of the tests noted here, except the gluconate reaction, is new, since it had been found that most of the newer tests, such as the production of various amino-acid decarboxylases and phenylalanine deaminase, so commonly used in studies on other coliform bacteria (cf. Cowan, 1956), would not distinguish between the various strains of soft-rot coliforms (Graham, unpublished). The gluconate test, which depends on the oxidation of gluconate to the reducing

substance, 2-ketogluconate, was used originally by Haynes (1951) to distinguish between various pseudomonads. Cowan (1955) showed it was a useful test for *Aerobacter* (*Klebsiella*), since almost all strains of *A. aerogenes* and *A. cloaceae* gave a positive reaction. We have confirmed this, and found it to be a simple, quick, practical method of differentiating between soft-rot coliforms and the very similar common saprophyte *A. cloaceae*, since the soft-rot coliforms invariably give a negative reaction.

TABLE 1. *Biochemical reactions of organisms used in pathogenicity tests*

	Strain reference number												
Biochemical test	SR2/1	HT/1	QB4	123	14B	340	312	377	H2	EP3	370	A81V	398
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+
Pectate gel	+	+	+	+	+	+	+	+	+	+	+	+	+
Gelatin	+	+	+	+	+	+	+	+	+	+	+	+	+
Indole	−	−	−	−	−	−	−	+	+	+	−	−	+
Hydrogen sulphide	−	−	−	+	+	−	+	−	+	+	+	−	+
Gluconate test	−	−	−	−	−	−	−	−	−	−	−	−	−
Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+	+	+
M.R.	+	+	+	−	−	−	+	−	−	−	−	−	−
V.–P.	−	−	−	+	+	+	−	+	+	+	+	+	+
Gas from glucose	+	+	+	−	+	+	+	+	−	+	−	−	+
Acid from glucose	+	+	+	+	+	+	+	+	+	+	+	+	+
Acid from sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+
Acid from lactose	+	+	+	+	+	+	+	+	+	(+)	+	+	(+)
Acid from maltose	+	+	+	+	−	−	+	+	−	−	−	−	−
Acid from salicin	+	+	+	+	+	+	+	+	+	+	+	+	+
Acid in ethyl alcohol agar	−	−	−	+	+	−	+	+	−	+	+	−	−

	399	402	39	H25	H35	H904	C1	C5	119V	B1	74V	1172
Motility	+	+	+	+	+	+	+	+	+	+	+	+
Pectate gel	+	+	+	+	+	+	+	+	+	+	+	+
Gelatin	+	+	+	+	+	+	+	+	+	+	+	+
Indole	+	+	−	−	−	−	−	−	−	−	−	+
Hydrogen sulphide	+	+	−	−	+	+	−	−	−	−	−	+
Gluconate test	−	−	−	−	−	−	−	−	−	−	−	−
Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+	+
M.R.	−	−	+	−	−	+	+	+	+	+	+	−
V.–P.	+	+	−	+	+	−	−	−	−	−	−	+
Gas from glucose	+	+	+	+	+	+	+	+	−	+	−	+
Acid from glucose	+	+	+	+	+	+	+	+	+	+	+	+
Acid from sucrose	+	+	+	+	+	+	+	+	+	+	+	+
Acid from lactose	(+)	(+)	+	+	+	+	+	+	+	+	+	+
Acid from maltose	−	−	+	+	+	−	+	+	−	+	−	+
Acid from salicin	+	+	+	+	+	+	+	+	+	+	+	+
Acid in ethyl alcohol agar	−	−	−	−	+	−	−	−	−	−	−	+

In gelatin row and pectate gel row; + = liquefaction.
In nitrate row; + = nitrites present.
In lactose row; (+) = acid production delayed at least 4 days.

Failure to produce small amounts of gas from sugars was confined to those cultures considered to be *P. carotovorum* var. *aroideae*. Although not a consistent biochemical reaction, since many gas-forming strains are well known to lose this ability after about a year in culture, we still consider that it is of use as a practical diagnostic characteristic when identifying newly isolated cultures.

Variable results have been reported for the methyl red and Voges–Proskauer tests, no doubt due to the use of different media and times of incubation before testing. For example, Burkholder & Smith (1949) reported the reactions of *P. carotovorum* and *P. carotovorum* var. *atrosepticum* as M.R. positive, V.–P. negative, when grown in ordinary glucose–phosphate–peptone for an unspecified number of days at 27° C.

On the other hand, Jones (1950) reported that all strains of soft-rot coliform bacteria were M.R. negative, V.–P. positive, but he preferred to use O'Meara's (1931) fumarate medium, incubating at 25° C. for 5 days. Taylor (1951) studied the M.R. and V.–P. reactions of eighteen soft-rot isolates, comparing ordinary glucose–phosphate–peptone with a medium claimed to be superior by Smith, Gordon & Clark (1946), and also O'Meara's fumarate medium. He concluded that the Smith, Gordon & Clark medium was useless for tests with soft-rot organisms, while with regard to the fumarate medium he considered that 'reliance should not be placed on its exclusive use, as was done by Jones (1950) or a valuable differential criterion may be lost', because the presence of fumarate favoured the production of acetylmethylcarbinol too strongly. Our results in ordinary glucose–phosphate–peptone incubated for 5 days at 26° C. showed that while all strains of *P. carotovorum* var. *atrosepticum* were M.R. positive, V.–P. negative, the great majority of the other isolates were M.R. negative, V.–P. positive, although strains such as 312 and 119V behaved like var. *atrosepticum*.

It has long been known that the maltose fermentation reaction is a variable character among the soft-rot coliforms, and various workers have attempted to use this feature for separation of species. For example, Dowson (1941) stated that *P. carotovorum* did not ferment maltose, while *P. carotovorum* var. *atrosepticum* did so with the formation of acid and gas. It is now generally recognized that this is incorrect, and that maltose fermentation is of little value in bacterial characterization. This is confirmed by our results, but it is noteworthy that all the *P. carotovorum* var. *atrosepticum* isolates did produce acid from maltose, while of the other organisms only strains 123, 312, 377, H25, H35 and 1172 fermented maltose. A similar situation is found with regard to acid formation from ethyl alcohol. For instance, Dowson (1957) stated that both *P. carotovorum* and *P. carotovorum* var. *aroideae* formed acid in ethyl alcohol agar, while *P. carotovorum* var. *atrosepticum* did not. This is generally true, but there are certain inconsistencies, for strains H2, 340, A81V, 398, 399 and 402 which do not form acid in ethyl alcohol agar cannot be considered as forms of the variety *atrosepticum*. In further experiments we found that cultures which produced acid in ethyl alcohol agar also formed acid in 5% ethyl alcohol-peptone (Bacto) broth (Noble & Marshall, 1952), but the ethyl alcohol beef-extract peptone broth recommended as a differential medium by Burkholder & Smith (1949) gave variable results. Microtests (Clarke & Cowan, 1952; Cowan, 1953) using washed suspensions of organisms grown on meat infusion agar, were carried out in buffered 5% ethyl alcohol with bromothymol blue indicator. These showed that acid production in ethyl alcohol alone was perfectly correlated with acid production in ethyl alcohol agar and ethyl alcohol peptone broth which indicated that the acid was not formed from other substances present in these media.

Most strains produce acid from lactose rapidly, but there are a few notable exceptions which are slower in this respect; these are EP3, 398, 399 and 402. Late lactose fermenters such as *E. carotovora* f.sp. *parthenii* (Starr, 1947), (398), *E. chrysanthemi* (Burkholder, McFadden & Dimock, 1953), (399), and the organisms

causing slow wilt of carnation described by Lelliott (1956), (402), are now well known, but they are much less common than the rapid lactose fermenters. A similar organism isolated from *Philodendron* (EP3) was named *E. chrysanthemi* var. *philodendri* by Miller (1956).

CONCLUSION

Because of the variability of the biochemical reactions, isolates capable of producing potato black-leg at both low and high temperatures, or at higher temperatures only, cannot be distinguished from one another with absolute certainty by biochemical tests alone. However, the tests give an indication of potential pathogenic reactions. The distinguishing tests we tentatively propose are given in Table 2, together with a suggested division into biochemical varieties.

TABLE 2. *Biochemical classification of* Pectobacterium carotovorum *and varieties*

Organism	Gas from glucose	Acid from glucose	Acid from lactose	Acid from maltose	Acid from ethyl alcohol	M.R.	V.–P.	Indole
1. Anaerogenic group								
High-temperature organisms. *P. carotovorum* var. *aroideae*	–	+	+	d*	d	d*	d*	d*
2. Aerogenic group								
(i) Give delayed fermentation of lactose. Indole test invariably +ve (high-temperature organisms). *P. carotovorum* var. *chrysanthemi*	+	+	×	–	d	–	+	+
(ii) Give rapid fermentation of lactose.								
(*a*) Organisms usually M.R. –ve, V.–P. +ve; often produce acid from ethyl alcohol (high-temperature organisms). *P. carotovorum*	+	+	+	d	d	d*	d*	d*
(*b*) Organisms usually M.R. +ve, V.–P. –ve; never produce acid from ethyl alcohol (low-temperature organisms). *P. carotovorum* var. *atrosepticum*	+	+	+	+	–	+	–	–

Notation used is that of Kauffmann, Edwards & Ewing (1956), viz. + = positive 1–2 days; × = late and irregularly positive (i.e. acid production delayed at least 4 days); – = negative; d = different strains give consistently different reactions. Asterisk: in maltose column = most isolates are negative; in M.R. and V.–P. columns = most isolates are M.R. negative, V.–P. positive; in indole column = most isolates are indole negative.

Few of these suggestions are new, since we consider our findings generally support and reinforce the proposals already made by Dowson (1957). For example, var. *aroideae* is still separated on the basis of failure to produce gas from sugars when first isolated, irrespective of any of its other reactions, but we are well aware that this point of view is open to criticism. Such organisms can produce black-leg only at comparatively high temperatures. The low-temperature isolates correspond with var. *atrosepticum*, and are distinguished by their gas production, rapid fer-

mentation of lactose and maltose, M.R. and V.–P. reactions, and failure to give a positive indole test.

Our only new proposal is to alter the name of the chrysanthemum bacterial blight organism from *P. carotovorum* f.sp. *chrysanthemi* (Dowson, 1957), to *P. carotovorum* var. *chrysanthemi*, and include in this variety the carnation slow wilt organism, the guayule pathogen, and the organism causing bacterial leaf rot of *Philodendron*. This is done on the basis of the delayed lactose fermentation reaction, and it is noteworthy that we have found this delay to occur both in inorganic basal media and lactose-peptone media. Great stress is laid by some bacteriologists on the significance of this reaction, and in fact the genus *Paracolobactrum* Borman *et al.* has been established on this criterion; it still appears in Bergey's *Manual of Determinative Bacteriology* (7th edition, 1957). Although we cannot agree that separate genera can be erected on the basis of a single biochemical reaction, we think we are justified in creating a new variety, especially as this group of organisms also gives a positive test for indole. We feel that the diagnosis of soft-rot coliform bacteria should be based as far as practicable on biochemical reactions, and not on host range characteristics, especially as the host ranges of the numerous soft-rot variants have never been completely determined. This is understandable, since most plant pathologists do not have time or facilities for making large-scale inoculations on a range of host plants. Again, as we have shown in the experiments reported in Graham & Dowson (1960), it is not sufficient to draw conclusions from the results of a single pathogenicity trial carried out at any convenient but arbitrarily selected temperature. A range of temperatures should be employed, otherwise the results can be readily misinterpreted. Furthermore, experience has shown that naming an organism solely on the host specificity is commonly of little practical use, because it fails to take into account those from other host plants or from soil, avirulent strains, and those of unknown origin.

At this stage in the study of soft-rot coliforms it is essential to compare the biochemical reactions of as many organisms as possible, to establish firmly a practical biochemical classification on quantitative lines, and at the same time, to investigate the mechanism of pathogenicity.

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COPPICING EXPERIMENTS ON THE SPREAD AND CONTROL OF CACAO SWOLLEN-SHOOT DISEASE IN NIGERIA

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(With 2 Text-figures)

A coppicing technique was used to determine the incidence and distribution of latent and unrecognized infection around naturally occurring outbreaks of cacao swollen-shoot virus disease in Western Nigeria. All the apparently healthy trees within 30 yards of eighty outbreaks of various sizes were coppiced and most of the infected stumps which regenerated showed symptoms within a year.

The distribution of the infected stumps around thirty-five outbreaks studied in detail is expressed by the equation $\log_{10} I = a + bx$. I is the estimated intensity of infection in the coppiced stumps at distance x from the nearest infected tree removed at the time of coppicing. The constant a determines the height of the peak of the infection gradient and increases with outbreak size, whereas the slope of the gradient, determined by the negative constant b, is similar around all outbreaks.

The results are consistent with information on the movement of the mealybugs and spread of cacao swollen-shoot virus in outbreaks. Moreover, they indicate that outbreaks are controlled most economically by removing all obviously infected trees and adjacent apparently healthy ones. Control does not require the destruction of all the trees around outbreaks up to a distance of 30 yards as done previously, but can be achieved by felling fewer trees, the actual number depending on the size of the outbreak.

Introduction

Swollen-shoot virus disease of cacao is widespread in Ghana, Ivory Coast and Nigeria and eradication is the only practical control measure which can be adopted (Thresh, 1958*a*; Thresh & Tinsley, 1960). Experience has shown that when only those trees found with symptoms during the routine inspections are removed, control is unsatisfactory and uneconomic, resulting in the eradication of only a proportion of outbreaks, mainly the smaller ones. In larger outbreaks, where infected trees tend to be more numerous and scattered, they are often missed and remain as sources of infection.

The efficiency of the control measures is improved by removing both the obviously infected trees and adjacent apparently healthy ones, at least some of which are usually infected. Thus in Ghana, control was improved when apparently healthy trees in contact with infected ones were destroyed (Posnette, 1943; Anon., 1945; Hammond, 1957). In Nigeria, the general practice adopted in the past has been to remove all the symptomless trees within 30 yards of infected trees and

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though good control was often obtained in this way, an average of ten trees were destroyed for every one obviously infected (Lister & Thresh, 1957; Thresh, 1958*d*). The coppicing experiments described here were started to determine whether these relatively successful but expensive measures were justified, or whether comparable results could be achieved less drastically. The results have given new information on the pattern of virus spread and indicate that eradication methods should differ according to the size of outbreak treated.

Materials and methods

Healthy cacao was not available for statistically designed infection experiments and all the results were obtained on numerous naturally occurring outbreaks found in peasant farms between 1954 and 1956. Only the more readily accessible of the surveyed parts of Ibadan Province were considered, and each treated outbreak was completely surrounded by at least 30 yards of cacao. Otherwise, the selection of outbreaks was at random and included farms in various localities, differing in age, condition and in the amount of damage due to other pests and diseases. Most infected trees showed swellings and leaf symptoms, but some showed only swellings or leaf mosaic. This suggests that a number of different virus strains were involved, a conclusion substantiated by the results of transmission tests. The outbreaks also differed in size, most involving fewer than twenty trees, although some involved more than one hundred. Such variation caused great differences between the results obtained. However, a sufficiently large number of outbreaks was treated to overcome this disadvantage and the results are representative of those to be expected over a wide area and over a range of size of outbreak.

All the treatments were done by experienced survey officers of the Department of Agriculture. Infected farms were first thoroughly examined and the trees with symptoms were marked and subsequently felled by severing the trunk immediately below ground level. The site of each infection was then marked with a painted wooden stake. In some outbreaks the apparently healthy trees immediately adjacent to the obviously infected ones were also destroyed. On all plots the remaining trees within 30 yards of the outbreaks were cut back to a height of 6 or 9 in. above ground level. Most trees coppiced in this way produced vigorous shoots, which were examined for virus symptoms during the routine inspection of the cacao around each treated area, usually every 3 months. The infected stumps found at each site were recorded at each inspection. In forty-eight of the outbreaks the distribution of the infected stumps in relation to the trees removed at the outset was recorded. Healthy shoots were thinned out, sprayed with insecticide to prevent damage by capsids, and their bases earthed up to facilitate rooting. The most satisfactory shoots bore pods in the second or third year after coppicing and had then developed their own root system and a closed canopy.

Results

The appearance of shoots with symptoms on coppiced stumps

Affected shoots developing from the stumps of infected trees are usually more conspicuous and accessible than those on intact trees. Indeed, coppicing is the most convenient of the methods available for demonstrating virus infection and provides an inexpensive means for the large-scale diagnosis of infected trees. Also most of the mealybugs on felled trees die without moving to others (Strickland, 1951*a*; Cornwell, 1956); spread of virus through the canopy is impossible until considerable regrowth has taken place. Thus coppicing probably reveals infection only in those trees already infected when they were cut back. These included some trees missed by the survey parties or infected with mild viruses, but most of them were recently infected ones that had not previously shown symptoms (Thresh, 1958*d*).

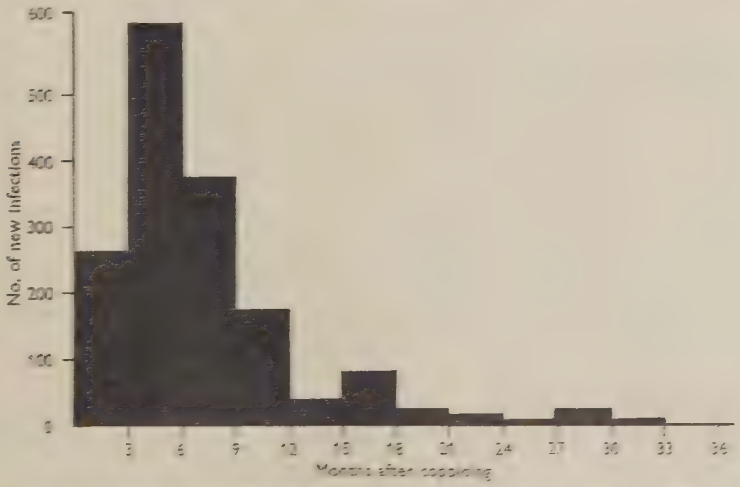

Fig. 1. The numbers of new infections identified in successive 3-month periods after coppicing around swollen-shoot outbreaks.

The only disadvantage of the coppicing technique was that one-third of the treated trees failed to regenerate. From other work it seemed that infected trees were less likely to regenerate than uninfected ones (Posnette & Todd, 1955; Thresh, 1958*b*), but the precise number of infected stumps lost in this way cannot be determined. However, the results of subsidiary experiments suggest that when infected trees are coppiced only half of them survive.

At the outset, coppicing was expected to reveal infected stumps rapidly. Actually most produced shoots with conspicuous leaf symptoms and/or swellings within a year, but some were detected only after 2 years (Fig. 1). These may have been infected after coppicing, by mealybugs blown from outside sources, but this is

unlikely, for their distribution was similar to that of the infected stumps that were identified quickly. Furthermore, equally long latent periods were observed in other experiments in which obviously infected trees were coppiced and the new growth examined (Thresh, 1958*b*).

The apparently long latent periods usually occurred with viruses that cause only transient leaf symptoms, which pass undetected unless they coincide with an inspection. Other stumps regenerated slowly, particularly when coppiced in the cool, cloudy periods of the rainy season (Thresh, 1958*b*). Shoots produced then were often temporarily leafless and at all seasons some showed conspicuous symptoms of iron, nitrogen and zinc deficiency, which mask those caused by viruses.

TABLE 1. *The incidence of latent and missed infections around outbreaks of cacao swollen-shoot disease where the contact trees were not destroyed*

	Mean number of			Ratio of		
Number of trees in outbreak*	Infections destroyed†	Apparently healthy trees coppiced	Infected stumps‡	Apparently healthy trees to infected	Infected stumps to original infections	Percentage stumps infected‡
> 50 (17)	141·9	1340·2	50·6	9·4:1	0·357:1	3·7
11–50 (19)	20·9	560·6	7·4	26·8:1	0·354:1	1·32
6–10 (12)	8·2	468·2	6·8	57·1:1	0·829:1	1·45
1–5 (32)	2·3	252·6	1·9	109·4:1	0·826:1	0·75
All outbreaks (80)	37·3	588·8	14·3	15·8:1	0·383:1	2·43

* The number of outbreaks in each category shown in brackets.

† The number of obviously infected trees found by the routine survey parties and removed at the time of coppicing.

‡ Revealed by coppicing the apparently healthy trees around each outbreak to a distance of 30 yards and keeping the new growth under regular symptom observation.

The incidence of latent and missed infections around virus outbreaks

In all analyses the results were arranged according to the number of infected trees originally found at each treated outbreak and the grouped data for the eighty outbreaks where 'contacts' were not destroyed are presented in Table 1. As previously stated, the actual figures for the incidence of infection in the coppiced stumps are probably underestimates, because of the mortality amongst the infected ones. This affects some of the ratios presented, but not the general conclusions, as there is no evidence that the survival of coppiced stumps is influenced by the size of outbreak treated.

The ratio of apparently healthy trees coppiced to the number of infected ones removed at the outset decreased with increasing outbreak size, a result to be expected from the observations made in Nigeria and elsewhere that outbreaks tend to be circular, as they spread radially outwards from the original source (Thresh, 1958*c*). By comparison, the number and proportion of infected stumps revealed by coppicing increased with outbreak size. However, even if the observed percentages of infected stumps are doubled (to allow for those that failed to regenerate) they remain low and the experiments suggest that 98·5% of all the coppiced trees around

the smallest outbreaks were healthy compared with 92·4% around the largest ones. Most of the stumps were probably of recently infected trees and the results emphasize that outbreaks become increasingly dangerous foci of infection as they spread unchecked. However, the ratio between the number of infected stumps and the number of originally infected trees was not constant, but was greatest in small outbreaks.

Results for the sixty-two outbreaks where the 'contact' trees were destroyed and not coppiced are presented in Table 2. The number of apparently healthy trees coppiced greatly exceeded the number destroyed, which was in turn greater than the number of infected trees found and uprooted. However, the ratios between the total number of apparently healthy trees and the obviously infected ones

TABLE 2. *The incidence of latent and missed infections around outbreaks of cacao swollen-shoot disease where the contact trees were destroyed*

	Mean number of				Ratio of		
Number of trees in outbreak*	Infections destroyed†	Apparently healthy		Infected stumps‡	Apparently healthy trees to infected	Infected stumps to original infections	Percentage stumps infected‡
		Destroyed	Coppiced				
> 50 (20)	258·7	453·4	1628·0	20·8	8·1 : 1	0·08 : 1	1·28
11–50 (19)	20·9	56·5	384·6	3·2	21·1 : 1	0·15 : 1	0·83
6–10 (7)	7·9	27·0	224·6	1·7	31·8 : 1	0·21 : 1	0·76
1–5 (16)	2·4	11·3	208·9	0·5	91·7 : 1	0·21 : 1	0·23
All outbreaks (62)	91·3	169·5	722·3	8·0	9·8 : 1	0·09 : 1	1·11

* The number of outbreaks in each category shown in brackets.

† The number of obviously infected trees found by the routine survey parties and removed at the time of coppicing.

‡ Revealed by coppicing the apparently healthy trees around each outbreak to a distance of 30 yards after the obvious infections and their contacts had been destroyed.

decreased with increasing outbreak size and were similar to those in Table 1. The removal of 'contacts' greatly reduced the number of infected stumps around outbreaks in each size category and the observed proportion of infected stumps was 1·11% compared with the 2·43% around outbreaks where contacts were not removed. This indicates that approximately half the latent and missed infections around an outbreak occur alongside obviously infected trees, a suggestion substantiated by the more detailed observations now presented.

The distribution of latent and missed infections around virus outbreaks

The distribution of infected stumps was recorded in thirty-five of the outbreaks where only obviously infected trees were destroyed. No infected stumps were found in seven, all of which contained fewer than six obviously infected trees. Elsewhere, 510 infected stumps were recorded and 51, 86, 95, 98, 99 and 100% occurred within 5, 10, 15, 20, 25 and 30 yards, respectively, of the originally uprooted infection. For example, the proportions of coppiced trees infected around a typical large outbreak at Ogundele were 77/312, 36/362, 3/412, 1/462, 0/512 and

0/565 in successive annuli, each 5 yards wide, around the 253 original infections. The distribution was similar around smaller outbreaks, but the observed intensities of infection were less and a smaller proportion of the apparently healthy trees were within 5 yards of the outbreak. Thus at Ajibope the corresponding proportions were 4/42, 1/84, 0/127, 0/168, 0/212 and 0/253 around an outbreak originally containing two obviously infected trees. The grouped data for the observed intensities of infection around outbreaks in each size category are presented in Fig. 2.

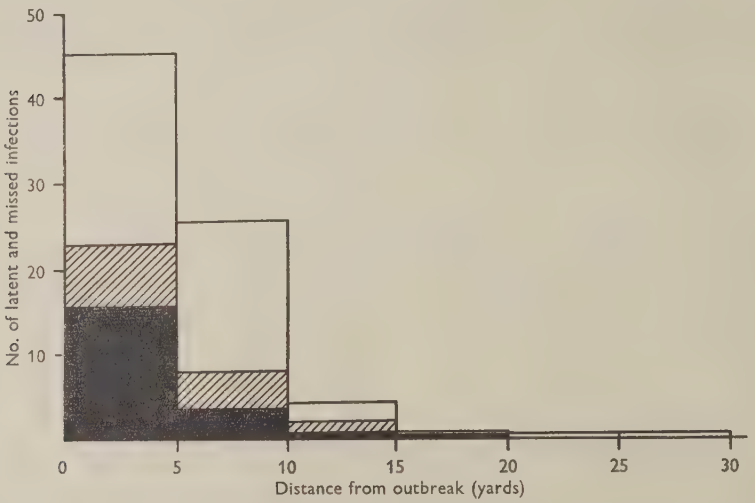

Fig. 2. The distribution of latent and missed infections around outbreaks of cacao swollen-shoot disease. Observed infection rates doubled to allow for the stumps which died, and transformed to allow for multiple infection (Gregory, 1947). ▩▨□, Pooled data for seven large outbreaks of > 50 obviously infected trees. ■▨, Pooled data for thirteen intermediate outbreaks of six to fifty obviously infected trees. ■, Pooled data for thirteen small outbreaks of one to five obviously infected trees.

With diseases of crops other than cacao, various relationships have been established for the occurrence of new infections with increasing distance from the source (Frampton, Linn & Hansing, 1942; Zentmeyer, Wallace & Horsfall, 1944; Wilson & Baker, 1946; Linn, 1940; Wolfenbarger, 1946). However, these data do not depart significantly from the simple relationship discussed by Gregory & Read (1949), and Bateman (1950), viz.

$$\log_{10} I = a + bxp,$$

where I = number of infective punctures at distance x from the source after any given time and a, b and p are constants for any one given set of field conditions.

The data presented in Fig. 2 also follow a leptokurtic distribution and can be expressed by a similar equation, where I = the observed percentage infection in the coppiced stumps, doubled to allow for the ones which failed to regenerate and

transformed to overcome the effects of multiple infection (Gregory, 1948). Y is the distance in yards from the nearest obviously infected tree destroyed at the outset. The constant a determining the height of the regression line increases with the size of the source, but the slope, determined by the constant b, is similar around all outbreaks of the size considered. For all outbreaks values of $p = 1$ fit the results more satisfactorily than $p = \frac{1}{2}$ or $p = 2$ and for the pooled data gave the highly significant regression coefficient of $-0{\cdot}984$.

Valid estimates of the probability of latent and missed infections occurring beyond the limits of the coppiced areas could not be made from the regression equations. Nevertheless, extrapolation indicates that long-range spread is not common and outlying infections are usually widely scattered and not found until virus has had time to spread from them to adjacent trees. Thus they occur as so-called 'satellite' outbreaks which tend to be more numerous around the larger outbreaks and decrease in frequency with distance from the primary source (Posnette, 1943; Thresh, 1958*b*, *c*).

Discussion

The spread of cacao swollen-shoot disease

Most of the detailed studies on the mealybug vectors of cacao swollen-shoot disease in West Africa have been on *Pseudococcus njalensis* Laing, the principal species in Ghana (Strickland, 1951*a*, *b*), and one of the commonest in Nigeria (Donald, 1955). This work has shown that adults are usually sedentary and cryptic in habit and unimportant in spreading virus. By comparison, the nymphs are relatively active, particularly during the first instar, when they are readily observed moving on the branches and from tree to tree through the canopy (Strickland, 1951*a*; Cornwell, 1958). They are also occasionally carried over greater distances by wind (Strickland, 1950; Cornwell, 1955).

The steep gradient in the numbers of latent and missed infections revealed by coppicing is in accord with the habits of the vector, although the discontinuous or so-called 'jump' spread of virus, presumably by wind-dispersed mealybugs, probably accounts for some infection in the contact trees and for most of the limited spread over greater distances. Perhaps unexpectedly there is no discontinuity in the infection gradient, suggesting that the spread of virus by canopy movement becomes progressively and not suddenly less important with increasing distance from the source. However, an alternative explanation is that both wind dispersal and canopy spread are determined by similar factors, and it is known that the activity of mealybug nymphs is correlated with air temperature (Cornwell, 1958).

These observations on mealybug movement are consistent with those on the pattern and distribution of virus spread (Thresh, 1958*c*) and with the data obtained in the coppicing experiments described here. For example, most of the mealybugs, on infected trees in small outbreaks, can reach healthy ones by walking limited distances through the canopy or by wind dispersal. Under these conditions the number of recent infections revealed by coppicing may equal or exceed the original number of obvious infections. By comparison, most of the mealybugs on infected

trees in a large outbreak can reach healthy ones only on the rare occasions when they are carried by wind currents and spread usually occurs from recently infected trees on the perimeter, particularly as these are likely to support higher mealybug populations than the less vigorous trees in the centre of the outbreak in a more advanced stage of infection (Cornwell, 1956). In these circumstances the rate of spread of virus from outbreaks to the surrounding healthy cacao declines in relation to the total number of existing infections. Moreover, the number of recently infected trees revealed by coppicing the apparently healthy ones surrounding outbreaks becomes progressively less, compared with the original numbers of obvious infections.

Our experiments suggest that swollen shoot is a 'crowd' disease of the type discussed by van der Plank (1948) and defined by him as not spreading far in any considerable amount. Various field observations also support this view; for example, virus spreads slowly into and within newly planted cacao until a closed canopy is formed and facilitates the movement of mealybugs (Benstead, 1953). Moreover, virus has not spread extensively from the abandoned areas in Ghana and Nigeria where infection has been widespread for many years (Lister & Thresh, 1957; Thresh, 1958*d*). Plots of healthy trees can be maintained even in the most heavily infested areas, provided they are not touching infected ones (Benstead, 1953). Indeed, one of the methods used for the control of virus in Ivory Coast is to remove only the trees on the perimeter of each outbreak and replace them by coffee or other non-susceptible crops. This emphasizes the possible value of barrier crops and intercropping recently suggested by Cornwell (1956).

The control of cacao swollen-shoot disease

The limited distribution of the few latent and missed infections around the smallest outbreaks explains why these are more readily eradicated than large ones (Posnette, 1943; Anon., 1945; Thresh, 1958*d*). Indeed, removing only the obviously infected trees at the outset would have immediately eradicated infection from nineteen of the eighty treated outbreaks and from a further seven had the apparently healthy trees within 5 yards also been destroyed.

Even around the largest outbreaks the incidence of infected stumps was rarely sufficiently high to justify the former practice of destroying all the apparently healthy trees within 30 yards, and because of the results described, the methods of swollen-shoot control in Nigeria have been modified. All obviously infected trees are now being destroyed together with the adjacent apparently healthy trees within 5, 10 and 15 yards of outbreaks containing 1–5, 6–50 and 51–200 infected trees, respectively. Where this is done the estimated proportion of infected trees on the immediate edge of the treated areas is less than 5%, with lower intensities at increasing distances from the source. For example, if the new cutting-out measures had been used for the treatment of the outbreaks in the present investigations, 465 of the 510 latent and missed infections would have been destroyed at the outset. The remaining 45 would have occurred around 18 of the 33 treated areas and involved only a small proportion of the perimeter trees. Consequently these

outlying infections are most conveniently and economically treated as separate outbreaks, which are readily eradicated when found during the routine re-inspection of the trees around the treated areas. In this way major savings in Government expenditure are envisaged and relatively few healthy trees are now being destroyed compared with the former system of treating all outbreaks to a uniform distance of 30 yards, irrespective of size or circumstance.

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THE USE OF A MULTIPLE-TRANSFER METHOD IN PLANT VIRUS TRANSMISSION STUDIES—SOME STATISTICAL POINTS ARISING IN THE ANALYSIS OF RESULTS

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(With 2 Text-figures)

The frequency of success of attempts to transmit a virus disease from one plant to another has long been used to measure the effects of any of the factors which influence transmission. Samples are taken from the population under test (e.g. vectors, diseased plants, etc.), and usually one sample is tested on each test plant; this is binomial sampling. However, in the procedure we name the 'multiple-transfer method' more than one sample may be tested on each test plant. This increases the number of samples tested without increasing the number of test plants used, and errors due to heterogeneity in the population under test are therefore minimized. Results from experiments using the multiple-transfer method may be evaluated by using the maximum likelihood estimator. The method is particularly reliable when the proportion of infected samples being studied is small, but can lead to considerable over-estimation when the proportion is high.

Introduction

Attempts to transmit a virus disease from one plant to another depend on many factors. The frequency of successful transmissions may be used as a measure of the effect of any one factor. For example, the frequency with which single aphids from a clone transmit a virus disease under standard conditions can be used to define the efficiency of aphids of that clone as vectors, and can be used to compare that clone with other clones (Doncaster & Kassanis, 1946; Watson & Roberts, 1939). Similarly, the frequency with which a virus disease can be transmitted under optimum standard conditions from samples of a plant population is an estimate of the proportion p of diseased plants in the population.

The simplest way to estimate the proportion p is to transmit the virus from one sample plant to one test plant and replicate this procedure a number of times. The proportion of test plants that become infected is then a direct measure of the proportion p. However, there may be only a few, unevenly dispersed, infected plants in the population and so this method becomes impracticable because a large number of replications will be needed to estimate p with any accuracy. To increase the number of samples tested without increasing the number of test plants used, we can test more than one sample on each test plant; this we call the multiple-transfer method. This method has previously been used by other workers, e.g. Bawden & Kassanis (1946); Doncaster & Kassanis (1946); Watson & Roberts (1939); Kirkpatrick & Ross (1952). The maximum likelihood estimator can then be used to estimate p from the results of such experiments. When p is low this method can

be expected to give good results, for when a test plant becomes infected it is likely that only one of the samples tested on it was infected, but when p is high more than one sample may have been infected and this uncertainty leads to a bias in the estimation of p. This difficulty has already been noted (Gregory, 1948) in interpretation of crop-infection counts. It appears, therefore, that the multiple-transfer method may be useful when p is low as it would allow more samples to be tested and so tend to reduce errors due to heterogeneity in the population under test.

We discuss below some of the properties of the maximum likelihood estimator, pointing out some dangers of the multiple-transfer method and indicating when it can be used to advantage. Examples are given to illustrate the use of the results obtained.

Properties of the maximum likelihood estimator

Let us consider a single experiment where:

N is the number of test plants used;

i is the number of sample plants tested on each test plant;

R is the number of test plants which become infected;

p is proportion of infected plants in the population being studied, and p^* is the maximum likelihood estimate of p;

$q = 1-p$ is proportion of uninfected plants in the population and $q^* = 1-p^*$ is the estimate of q.

The probability that none of i sample plants is infected is q^i. Therefore the probability that a test plant becomes infected when virus is transferred from i plants on to it is $1-q^i$. The estimate q^* is then given by

$$R/N = 1-q^{*i},$$

therefore

$$p^* = 1-q^* = 1-(1-R/N)^{1/i}. \qquad (1)$$

An elementary investigation of the values that p^* may take helps considerably in understanding the properties that can be derived by mathematically more complex methods. Except when $R = N$ (i.e. when $p^* = 1$) the highest estimable value of p^* is when $R = N-1$. Fig. 1 shows how this maximum value of p^* varies with N and i.

For each value of i, R may take the values 1, 2, 3... $(N-1)$ to give $(N-1)$ values of p^* lying between 0 and 1. In Fig. 1 we see that these $(N-1)$ values are concentrated over a smaller range for higher values of i than for lower values of i. Thus when $N = 50$ and $i = 8$ there are forty-nine possible values of p^* concentrated between 0 and 0·39, but when $N = 50$ and $i = 2$ the range is from 0 to 0·85. This implies that to get the best results we must choose values of N and i so that the possible values of p^* are concentrated about the likely value of p. Thus for low values of p, i should be high but for high values of p, i must be low. The dangers of using high i when p is high can be seen in the following example. Suppose $N = 50$, $i = 8$ and the true value of p is 0·7; then it is almost certain that either forty-nine or more probably all fifty test plants will become infected. The two values of p^* corresponding to these two possibilities are 1·0 and 0·39, respectively; both have an error of about 0·3.

The above results can be confirmed and put more precisely by evaluating the bias† and standard error of p^*. This has been done on the electronic computer at Rothamsted. The results for $N = 10$ and $N = 50$ are presented graphically in Fig. 2A and B, which shows that p^* is biased if i is greater than one, the bias increasing as i increases. Fig. 2A and B shows that when $i = 2$ the standard error of p^* is generally lower than that for simple binomial sampling (i.e. where $i = 1$), and only slightly higher for some high values of p, whilst throughout the range the

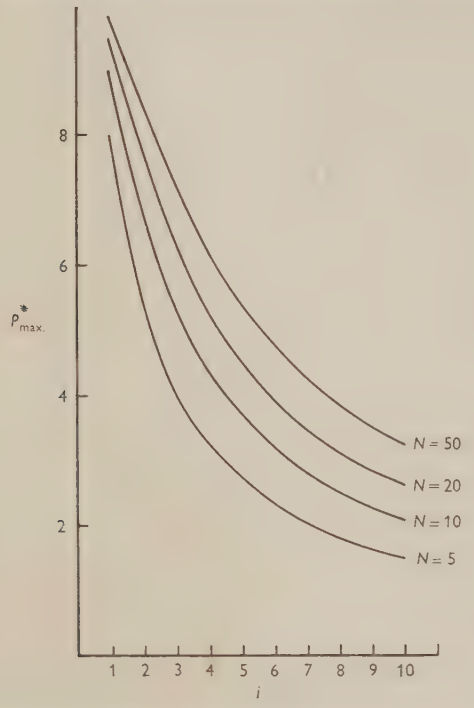

Fig. 1. The relationship of the maximum value of p^* (other than unity) estimable, with N and i.

bias is never large. For values of i greater than 2 there is a range of the higher values of p where both standard error and bias are so large that the estimate p^* becomes useless, and consequently these high values of i should be used with the greatest of care. Experimentally it is of great value to have some previous idea of the value of p, so that the best possible values of N and i can be used to keep errors in the estimate p^* to a minimum. To get this information it is advisable to do a preliminary trial with i equal to 1 or 2, and it is worth noticing that when $i = 2$ is taken there will be a slight tendency to overestimate p and that this will cause subsequent planning to err on the side of caution.

† A biased estimator is one such that, on repeated sampling, the average value of the estimate is not equal to the true value of the parameter being estimated.

Estimates can sometimes be corrected for bias but we have found no generally satisfactory method in this case. The following method is intuitively attractive and will be illustrated by an example. Suppose $N = 50$ and $i = 8$ and a sample has given $p^* = 0{\cdot}35$. A line is drawn between the values 0·35 on both axes of the $N = 50$ graph (Fig. 2B) and this line cuts the $i = 8$ bias curve at a point corresponding to $p^*_1 = 0{\cdot}30$ and this is taken to be the corrected value of p^*. The method is unsatisfactory for two reasons; firstly, a set of graphs corresponding to all values

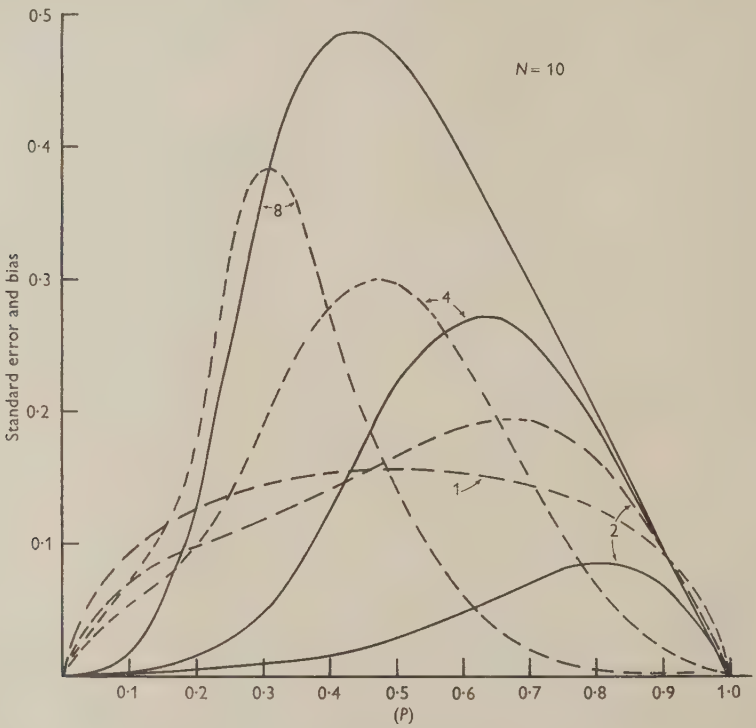

Fig. 2A. The variation of bias and standard error with p for $i = 1, 2, 4, 8$. $N = 10$. – – –, Standard error; —, bias.

of N and i likely to be used would have to be prepared, and secondly, these graphical estimates are still biased and may have a greater standard error than the original estimate, and when p is high the bias will almost certainly be increased.

An alternative method of bias correction can be obtained as follows. It can be shown that at low values of p the bias $b(p^*)$ of p^* is related to p in the following way

$$p - b(p^*) = [Ni\{1-(1-1/N)^{1/i}\}]p \qquad (2)$$

which implies that

$$p^*_2 = [Ni\{1-(1-1/N)^{1/i}\}]^{-1}p^*$$

should be a better estimate when p is small. Unfortunately, this correction is again not strictly valid for high values of p and leads to only insignificant corrections when p (and therefore also the bias) is small. Formula (2) shows that with i constant, bias is approximately halved when N is doubled.

When i becomes large (2) takes the following form

$$p - b(p^*) = -Np \log_e(1 - 1/N)$$

which shows that the relative bias varies from about $1/4N$ when $i = 2$ to $1/2N$ for large values of i.

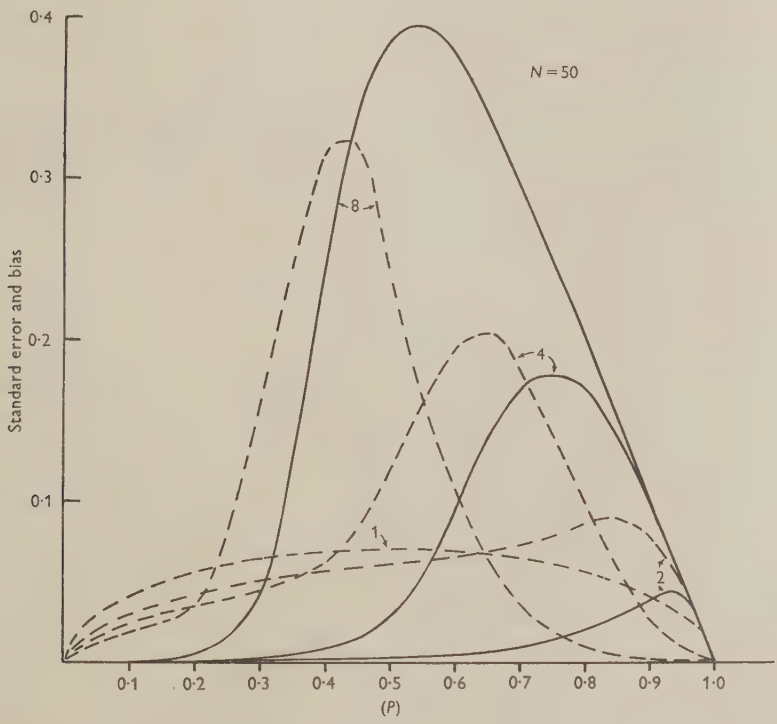

Fig. 2B. The variation of bias and standard error with p for $i = 1, 2, 4, 8$. $N = 50$. – – –, Standard error; —, bias.

Biological limitations of the method

In the mathematical treatment it has been assumed that if virus can be transmitted from one sample to a test plant when tested alone, then it can still be transmitted when tested together with one or more other samples on one test plant. In the examples given below experimental conditions have been chosen to make this

assumption justifiable. However, in other instances this may not be so; when samples of leaves are inoculated to test plants, sap from uninfected leaves may dilute that from an infected leaf to such an extent that the virus is not detected. Moreover, there is evidence (Kirkpatrick & Ross, 1952) that an infective aphid transmits virus more frequently when caged alone on a test plant than when caged together with other non-infective aphids. It has also been assumed that if virus is not transmitted from one sample to a test plant when tested alone, it will still not be transmitted when the sample is tested together with one or more similar samples, even though the samples may contain small amounts of virus.

Examples

Four examples will be given:

(1) An estimate was made of the incidence of lucerne mosaic virus in field lucerne (*Medicago sativa* L.).

Samples of lucerne were assayed for virus by rubbing sap extracted from them on to the primary leaves of French bean plants (*Phaseolus vulgaris* L. var. Prince): brown necrotic lesions of lucerne mosaic developed in 2–3 days on those leaves rubbed with sap from infected samples.

A preliminary survey showed that approximately 5% of the lucerne was infected. Fifty test plants were to be used in the experiment. Fig. 2B shows that if $p = 0{\cdot}05$ and $N = 50$ there is little bias for values of i up to 8, and standard error decreases as i increases. Therefore the value of i can be chosen by considering how many samples can be conveniently collected. It was decided to have $i = 4$, for it was thought that 200 plants would adequately represent the area being sampled, whereas increasing i to 8 would have doubled the work of collection of samples but not have decreased the standard error appreciably.

Eight of the fifty test plants became infected. By use of formula (1) we find that $p^* = 0{\cdot}0425$, in other words an estimated 4·25% of the lucerne was infected. The estimated standard error given by Fig. 1 is 0·01 or 1%.

(2) The incidence of sugar beet yellows virus (SBYV) was compared in wild beet (*Beta maritima* L.) from two foreshore sites in Suffolk.

The SBYV was transmitted from single leaf samples of the wild beet by apterous *Myzus persicae* (Sulz.) to healthy 'Klein E' sugar-beet seedlings in the glasshouse. The duration of both the acquisition and the test-feeding periods was 24 hr., and ten aphids were used for each transmission. After the test feed the test plants were sprayed with insecticide and kept in an aphid-free glasshouse for symptoms to develop.

The wild beet at the two sites were of different ages; most of those at Harkstead were more than 1 year old, whereas all the Stutton plants were less than 1 year old. The experiment was done to assess the incidence of virus in wild beet of different ages.

Leaves of wild beet readily become yellow for a variety of reasons: thus it is difficult to assess the approximate incidence of SBYV in wild beet with any certainty. Therefore virus estimates were done using different values of i with

constant $N = 10$. Four groups of ten test plants were used for the samples from each site, the groups being with $i = 1, 2, 4$ and 8.

The results are set out in Table 1.

We can see from Fig. 2A that both the Harkstead results for $i = 1$ and 2 have low bias and similar standard errors, and are likely to be equally good estimates of p. If p is between 0·70 and 0·55 then we can see from Fig. 2A that estimates of p using $i = 4$ or 8 are likely to be very seriously biased and have high standard errors,

TABLE 1. *Estimation of the incidence of SBYV in wild beet*

	Harkstead (old beet)				Stutton (young beet)			
N	10	10	10	10	10	10	10	10
i	1	2	4	8	1	2	4	8
R	7	8	9	10	1	3	6	8
p^*	0·70	0·55	(0·44)	(1·0)	0·10	0·16	0·20	(0·18)

and should be discarded. A combined estimate of p can be derived by weighting the observed values of p^* by estimated inverse variances as obtained from Fig. 2. This gives

$$p^* = \frac{p_1^*/s_1^2 + p_2^*/s_2^2 + \ldots + p_n^*/s_n^2}{1/s_1^2 + 1/s_2^2 + \ldots + 1/s_n^2}$$

$$= 0{\cdot}64.$$

The standard error is

$$(1/s_1^2 + 1/s_2^2 \ldots 1/s_n^2)^{-\frac{1}{2}}.$$

This result compares with $p = 0{\cdot}62$, the value obtained by the full maximum likelihood estimate using the results for $i = 1$ and 2 simultaneously.

The Stutton wild beet estimates are all between 0·10 and 0·20. Fig. 2A shows that only the estimate where $i = 8$ is likely to be inaccurate enough to be discarded. The mean estimate may be calculated from the other three estimates as above to give $p^* = 0{\cdot}15$ or 15% infection.

These results compare well with the visual estimates of 44 and 12% virus yellows at the same two sites, but suggest that some of the infected plants were not showing clear symptoms.

TABLE 2. *The efficiency of aphid species as vectors of SBYV*

	M. persicae				*M. ascolonicus*				*A. fabae*			
N	10	10	10	10	10	10	10	10	10	10	10	10
i	1	2	4	8	1	2	4	8	1	2	4	8
R	3	7	10	10	3	5	5	7	1	3	2	6
p^*	0·30	0·45	(1·0)	(1·0)	0·30	0·29	(0·15)	(0·04)	0·10	0·16	0·05	0·11

(3) The use of these methods is not confined to the estimation of disease incidence, but can be used to estimate the effect of any factor that influences virus transmission. The third example is of an experiment to compare the efficiency of clones of different aphid species as virus vectors. The probability with which a single aphid of each species would transmit the virus was determined by varying the number of aphids

per transfer (i). Clones of *Myzus persicae* (Sulz.), *M. ascolonicus* (Doncaster) and *Aphis fabae* (Scop.), were used to transmit SBYV from sugar beet to sugar beet (Table 2). As in the last example the duration of both the acquisition and test feeds was 24 hr.

By use of Fig. 2A and considerations similar to those used in example 2 we may discard the results for *M. persicae* and *M. ascolonicus* with $i = 4$ and 8. The mean estimates are then calculated for the three species as in example 2.

	M. persicae	*M. ascolonicus*	*A. fabae*
p^*	$0{\cdot}37 \pm 0{\cdot}102$ (37 %)	$0{\cdot}29 \pm 0{\cdot}091$ (29 %)	$0{\cdot}069 \pm 0{\cdot}026$ (6·9 %)

This shows clearly the different efficiencies of the three aphid species. As the transmission of a virus by aphids depends on many factors, most of which will vary between experiments (Kleczkowski & Watson, 1944; Watson, 1936, 1940), these results are of use only to determine the relative and not the absolute efficiencies of the aphid species as vectors. The results above show that *M. persicae* transmits SBYV about one and a half times as readily as *M. ascolonicus*, which is four times as efficient as *A. fabae*. This result compares with the experiments of Doncaster & Kassanis (1946) from whose results calculations show

	N	i	R	p^*
M. persicae	5	20	5	1
M. ascolonicus	11	10	5	0·06

The result for *M. persicae* is likely to be extremely inaccurate and is not inconsistent with a true value of p between 0·1 and 1·0, whereas the result for *M. ascolonicus* has given a reasonably accurate estimate due to a happy choice of N and i.

TABLE 3

	Varieties					
	Arran Banner		Majestic		Arran Consul	
N	36	36	36	36	36	36
i	2	4	2	4	2	4
R	3	10	5	17	10	19
p^*	0·04	0·08	0·07	0·15	0·15	0·17
	Combined					
p^*	0·06		0·10		0·16	

	Varieties					
	Arran Pilot		May Queen		Tobacco	
N	36	36	36	36	36	36
i	2	4	2	4	2	4
R	12	25	15	21	25	36
p^*	0·19	0·26	0·24	0·20	0·45	(1·0)
	Combined					
p^*	0·22		0·21		0·45	

It can be seen that with only slightly different results the estimated relative efficiencies of the aphids as vectors would be reversed.

(4) It is also possible to measure and compare the susceptibility of plants to an aphid-transmitted virus by use of the multiple-transfer method. The susceptibility of the plant can be expressed as the probability with which one aphid would infect it. Bawden & Kassanis (1946) give results of experiments in which different numbers of aphids were used to transmit potato virus *Y*. From these results can be calculated the probability with which one aphid transmitted the virus to the different varieties of potato. These results are set out in Table 3.

We can see from Fig. 2A and B that only the result for tobacco with $i = 4$ is likely to be inaccurate enough to be discarded. The results using two aphids per transfer ($i = 2$) show larger differences between varieties in the number of infected plants than when $i = 4$. However, the estimate p^* for $i = 2$ is slightly less accurate than when $i = 4$, as, firstly, the possible results when $i = 2$ are spread from 0 to 0·82 whilst when $i = 4$ they are concentrated between 0 and 0·575 (Fig. 1), and secondly, the standard errors for $i = 4$ are slightly lower if p is less than about 0·3. Therefore p^* is most accurately estimated from the results of the transmissions using four aphids per transfer.

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THE TOXICITY OF ZINC SULPHATE TO RAINBOW TROUT

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(With 5 Text-figures)

The toxicity of zinc sulphate to rainbow trout (*Salmo gairdnerii* Richardson) has been investigated in waters of different chemical and physical properties.

Zinc sulphate was less toxic to rainbow trout in hard water than in soft water; when the log concentration of zinc was plotted against log median period of survival of the fish the dose response curve was linear in a very soft water, and curvilinear in a hard water, approaching an apparent threshold concentration. Solutions of zinc sulphate containing calcium chloride were less toxic than those containing an equivalent concentration of calcium as bicarbonate.

An increase in temperature decreased the survival time of rainbow trout in solutions of zinc sulphate in a hard water, but the threshold concentration was not appreciably affected by changes in temperature.

A reduction in the dissolved oxygen concentration of the water increased the toxicity of zinc sulphate, but the effect was reduced when the fish were previously acclimatized to the lower oxygen concentration of the test.

The cause of death of fish in solutions of zinc sulphate was not by the precipitation of mucus on the gills but probably by damage to the gill epithelium.

Introduction

Published data on the toxicity of zinc salts to fresh-water fish have been reviewed by Doudoroff & Katz (1953) and McKee (1952, 1954) and show wide variations in the concentrations quoted as toxic. The different species of fish used by various investigators will have caused some of this variety, but much will have been due to differences in the chemical and physical properties of the dilution waters used, as it is known, for example, that zinc is less toxic in a hard water than in a soft (Jones, 1938). Although Cairns & Scheier (1957) have published 96 hr. TL_m* figures for zinc, using the common bluegill, in a hard and a soft water, the relation between period of survival and concentration of zinc has never received detailed investigation in waters of different hardness values. Other factors likely to influence the toxicity of zinc, but which do not seem to have been investigated in detail, are the temperature and the concentration of dissolved oxygen, and the work described in this paper was undertaken to examine the effects of these variables on the toxicity of zinc to the rainbow trout (*Salmo gairdnerii* Richardson).

* 96 hr. TL_m is the concentration at which 50 % of a batch of fish are killed after 96 hr. immersion.

Methods

The experiments were made with fixed volumes of solution in 40 l. aquaria fitted with thermostatic control. Dissolved-oxygen concentrations were maintained by aerating with diffused air at 4 l./min. when a concentration equal to the air-saturation value was required, and with mixtures of air and nitrogen to obtain lower concentrations. The pH values of the solutions were controlled by adding a small measured flow of carbon dioxide to the gas supply; the maximum concentration of carbon dioxide maintained in solution was 16 p.p.m., which is well below the toxic level in fully aerated water (Alabaster, Herbert & Hemens, 1957). Gas flows were controlled by a system of pressure-reducing valves, needle valves, and calibrated flowmeters; variations in the concentration of dissolved oxygen were not more than $\pm 0{\cdot}2$ p.p.m., and variations in pH values did not exceed $\pm 0{\cdot}15$ units.

The majority of the tests were made in water pumped directly from a borehole to the Laboratory; it had the following analysis: total hardness 320 p.p.m. as $CaCO_3$; total alkalinity to methyl orange 240 p.p.m. as $CaCO_3$; calcium 120 p.p.m.; magnesium 5 p.p.m.; sulphate 35 p.p.m.; chloride 19 p.p.m.

This water was also supplied to the aquaria in which the stocks of fish were kept. Artificial soft waters were prepared by diluting the borehole water with demineralized water obtained by passing the borehole water through a cationic exchange resin followed by an anionic exchange resin; 15 p.p.m. sodium chloride was then added to the water. Very soft waters (12 p.p.m. as $CaCO_3$) to which no sodium chloride had been added were slightly toxic to unfed rainbow trout; the osmotic pressure of the blood of trout exposed to this water, measured by the depression of the freezing point, showed a definite drop in 96 hr., but the addition of 15 p.p.m. sodium chloride, a concentration about that which is usually found in natural soft waters (Taylor, 1958), made it non-toxic, and in such water no fall in the osmotic pressure of the blood was observed.

Zinc was added to the test solutions in a few initial tests as zinc chloride and as zinc sulphate in later experiments; there was no difference in the toxicities of these two salts. The concentration of zinc in solution was determined once each day by the mixed colour dithizone method (Sandell, 1950); there was no detectable loss of zinc from solution in tests lasting 24 hr.

Most of the rainbow trout were obtained from a commercial trout farm supplied with a hard water (280 p.p.m. as $CaCO_3$) and kept in stock tanks at the laboratory. For one series of experiments, rainbow trout were obtained from another farm with a relatively soft water supply (50 p.p.m. as $CaCO_3$) and were kept in the laboratory in an artificial soft water. The age of the rainbow trout used varied from 3 to 15 months, but all comparative experiments were made with fish of the same age. For each experiment, the fish were sorted at random into batches of ten for acclimatization, the duration of which varied with the test. They were transferred to fresh solutions daily if the test continued for more than 24 hr. Fish were not fed for 24 hr. before testing nor during the test itself, unless it continued for more than 3 days, in which case they were fed every third day. The period of survival of a

fish was taken from the time of immersion in the solution to the time when all respiratory movement had ceased; the distribution of individual survival times was usually log-normal. Median periods of survival were calculated graphically (Bliss, 1937) from the individual survival times in tests in which 50% or more of the fish died within the experimental period.

Results

Toxicity of zinc in waters of different hardness

(*a*) *Hard water*

The solubility of zinc decreases with an increase in pH value, but, except where specifically stated, all the zinc was in solution in the experiments described here. The relation between the logarithm of the concentration of zinc and the logarithm of the median period of survival of rainbow trout in a hard water (320 p.p.m. as

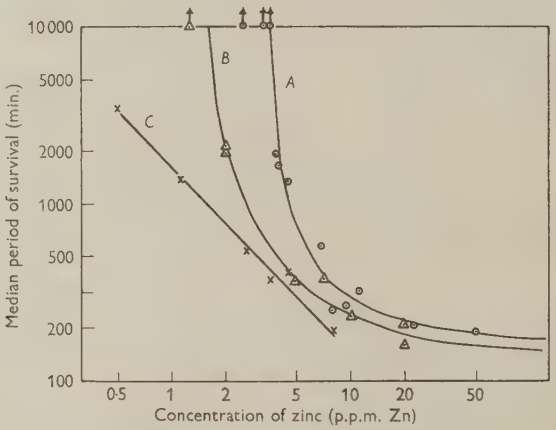

Fig. 1. Toxicity of zinc to rainbow trout in waters with different total hardness values. *A*, total hardness 320 p.p.m. as $CaCO_3$, pH 7·6–7·8. *B*, total hardness 50 p.p.m. as $CaCO_3$, pH 7·0–7·2. *C*, total hardness 12 p.p.m. as $CaCO_3$, pH 6·6–6·7.

$CaCO_3$), temperature 17·5° C., and pH value 7·8, is shown in Fig. 1, line *A*; this relationship is curvilinear and with decreasing concentration the curve appears to approach a threshold concentration below which zinc is not toxic. In common with most poisons there is also a threshold reaction time which cannot be much reduced by a further increase in the concentration of zinc; this period, about 160 min. under these experimental conditions, is longer than that found for other poisons investigated by this laboratory.

To determine the period for which rainbow trout could be exposed to a highly toxic concentration of zinc salt before the toxic effect became irreversible, four batches of ten fish were immersed in solutions containing 20 p.p.m. zinc; a batch was transferred to clean water every 40 min. Both the batches removed at 40 and

80 min. survived for at least 3 days, whereas 50% of the batch removed after 120 min. immersion died. The median period of survival of the fourth batch of rainbow trout, which were not removed from the zinc solution, was 150 min. It is evident, therefore, that for at least half of the long threshold reaction time the action of zinc is not irreversibly toxic.

There is evidence that fish exposed to concentrations of zinc below the threshold value acquire an increased resistance to higher, lethal, concentrations. Thus, at the end of the experiment represented by curve *A*, Fig. 1, two batches of fish had survived in solutions containing 2·5 and 3·5 p.p.m. zinc for 14 days. These fish, together with the control batch, were exposed to solutions containing 10 p.p.m. zinc at the same temperature and pH value as those at which they had previously been tested. The median period of survival of fish acclimatized to 3·5 p.p.m. was 500 min., compared with 400 min. for fish previously exposed to 2·5 p.p.m. zinc, and 290 min. for fish not acclimatized; the median period of survival was significantly longer ($P < 0{\cdot}001$) with an increase in the sublethal concentration of zinc in which they had previously been kept.

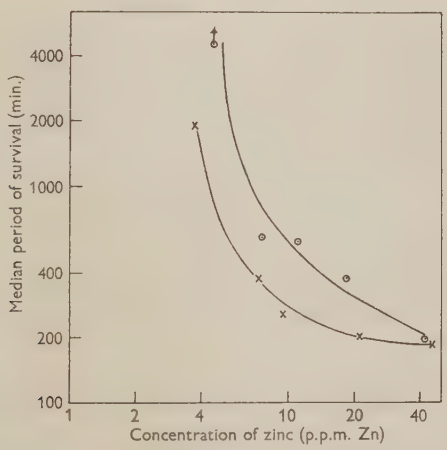

Fig. 2. Relation between concentration of zinc and period of survival of rainbow trout in water containing calcium bicarbonate and in water containing the equivalent concentration of calcium as chloride. ×—×, Calcium bicarbonate; ⊙—⊙, calcium chloride.

TABLE 1. *Toxicity of suspensions of precipitated zinc to rainbow trout in hard water* (320 *p.p.m. as* $CaCO_3$). *Temperature* 15° C.

Total concentration of zinc (p.p.m.)	pH value	Zinc in suspension (p.p.m.)	Zinc in solution (p.p.m.)	Median period of survival (min.)
10	7·97	Nil	10	285
20	7·96	9·0	11	180
30	7·94	18·5	11·5	162

Median periods of survival differ significantly at the level $P < 0{\cdot}001$

At pH values above 8·0, zinc becomes increasingly insoluble in hard waters until at pH 8·5 the amount held in solution is about equal to the threshold concentration for rainbow trout. It appears, however, that precipitated zinc compounds are toxic; Table 1 shows the results of an experiment in which a suspension of the zinc compound formed in hard water reduced the survival time of rainbow trout in solutions containing approximately the same concentration of zinc in solution.

In the experiments described so far, the hardness of the water was largely temporary or bicarbonate hardness. Since Jones (1938) had investigated the effect of increasing the hardness of the water on the toxicity of lead and zinc by adding calcium chloride to a soft water, an experiment was made to compare the toxicity of zinc in both a dilution water in which the majority of the calcium was present as the bicarbonate (borehole water) and also in borehole water which had been treated with hydrochloric acid to replace the bicarbonate by chloride, and a mixture of sodium hydroxide and carbonate added to restore the pH value. The results of this are shown in Fig. 2; zinc was markedly less toxic in water containing calcium chloride than in water containing calcium bicarbonate.

(*b*) *Soft water*

Rainbow trout, reared in soft water and kept in an artificial soft water (total hardness 12 p.p.m. as $CaCO_3$) at the laboratory, were acclimatized to the pH value (6·5) and temperature (17·5° C.) of the test before exposure to solutions of zinc. The relation between the logarithm of the median period of survival and the logarithm of the concentration of zinc (Fig. 1, line *C*) appears to be linear in these conditions.

Other rainbow trout from the same stock were kept in an artificial soft water with a total hardness of 50 p.p.m. as $CaCO_3$. They were acclimatized to temperature (17·5° C.) for 5 days, and to pH value (7·15) on the last day before exposure to solutions of zinc. In this dilution water there is a curvilinear relation between the logarithm of the median period of survival and the logarithm of the concentration of zinc (Fig. 1, line *B*).

The form of the concentration–survival time relation

Where the relation between the logarithm of the concentration of poison and the logarithm of the median period of survival appears linear, the results can be expressed as Ostwald's equation (1907),

$$C^n T = K, \tag{1}$$

where C is the concentration of poison; T is the median period of survival; n, K are constants.

For line *C*, Fig. 1, the constants take the values of $n = 1{\cdot}064$ and $K = 1648$ min. In conditions where the relation is curvilinear, Wuhrmann (1952) has used the equation

$$(C-C_s)^n (T-T_s) = K, \tag{2}$$

where C_s is the threshold concentration of poison; T_s is the threshold reaction time; C, T, n, K are as in equation (1).

Approximate values for the threshold concentrations and threshold reaction time were obtained by extrapolating curves fitted by eye to the experimental points and then making iterative adjustments until a reasonably linear relation was obtained between log $(C-C_s)$ and log $(T-T_s)$. For curves *A* and *B*, the values for the constants are

Constant	Total hardness (p.p.m. $CaCO_3$)	
	320 (curve *A*)	50 (curve *B*)
C_s	3·5 p.p.m. Zn	1·5 p.p.m. Zn
T_s	160 min.	140 min.
n	1·24	1·43
K	813 min.	933 min.

The curves corresponding to these parameters are fitted to the experimental data in Fig. 1.

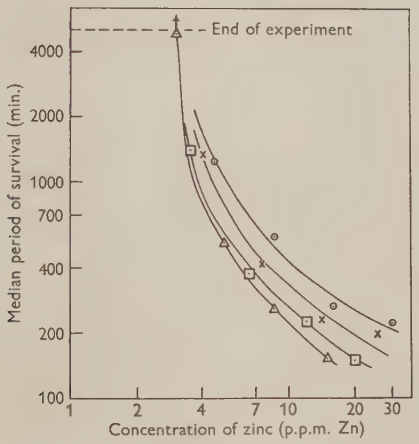

Fig. 3. Effect of temperature on the toxicity of zinc sulphate to rainbow trout. ⊙, 13·5° C.; ×, 15·5° C.; ⊡, 18·5° C.; △, 21·5° C.

Effect of temperature

Four batches of fish previously acclimatized to 13·5, 15·5, 18·5 and 21·5° C., respectively, for 5 days in borehole water were exposed to solutions of zinc at these temperatures and at pH values between 7·6 and 7·8. Results (Fig. 3) show that although the reaction time for a given concentration of zinc is increased by a reduction in temperature, the threshold concentration remains much the same. The relation between temperature and the logarithm of the median period of survival can be interpolated from the curves fitted by eye to the experimental points in Fig. 3; a straight line fitted to these points (Fig. 4) shows that a rise in temperature from 12 to 22° C. (i.e. 10° C.) reduces the survival time by a factor of 2·35.

Effect of concentration of dissolved oxygen

Tests were made in hard water at three non-lethal levels of dissolved oxygen, 8·9, 6·2 and 3·8 p.p.m. O_2 at 17·5° C., pH value 7·8. The rainbow trout were acclimatized for 18 hr. to the temperature and pH value, but not to the oxygen tension. Periods of survival of the fish in solutions of zinc are given in Fig. 5*a*;

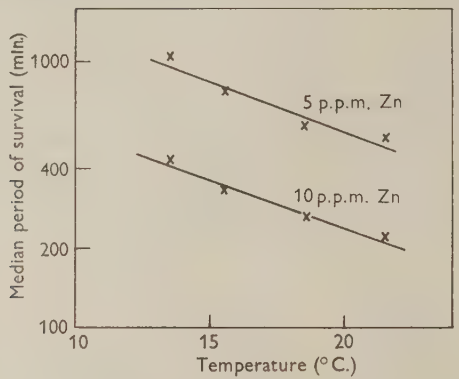

Fig. 4. Relation between temperature and period of survival of rainbow trout in solutions of zinc sulphate. Points interpolated from Fig. 3.

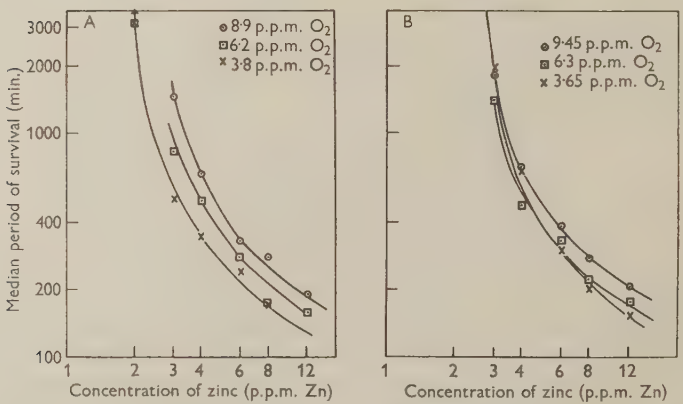

Fig. 5. Effect of the concentration of dissolved oxygen on the toxicity of zinc sulphate to rainbow trout. Temperature 17·5° C., pH 7·8 ± 0·2. A, Without acclimatization to dissolved oxygen concentration. B, With acclimatization to dissolved oxygen concentration for 18 hr.

a reduction in the oxygen concentration of the water increased the toxicity of zinc. A further experiment was made immediately with rainbow trout taken from the same stock tank, but with the fish acclimatized to the dissolved oxygen levels

(9·45, 6·3 and 3·65 p.p.m. O_2) at which they were to be tested, as well as to the temperature and pH value of the previous test for 18 hr. before they were subjected to zinc solutions. Results show (Fig. 5*b*) that the concentration of dissolved oxygen had less effect than in the previous experiment.

Site of toxic action of zinc

In the majority of the experiments described here, no precipitation of mucus was observed on the gills or in the gill chamber. On occasions when some of the zinc was precipitated from solution and was present as a suspension, white strands of mucus were observed to be attached to the gills; when the concentration of zinc in solution was high (above 15 p.p.m.), some mucus appeared to be precipitated over the surface of the body, but not in the region of the gills. It seemed unlikely, therefore, that in these experiments, the rainbow trout were dying from asphyxiation caused by coagulation of mucus on the gill surface, which has been reported by other workers (Carpenter, 1927; Ellis, 1937; Jones, 1938; Westfall, 1945) as the cause of death in solutions of metal salts. The matter was investigated in more detail by exposing a fish to a solution containing 20 p.p.m. zinc until it died and analysing the gills from each side of the fish for zinc. One set of gills was washed vigorously with a jet of distilled water to remove any possible traces of mucus, the other set was very gently rinsed in distilled water to remove traces of the solution of zinc; the zinc concentrations in the two preparations were 1·405 and 1·265 mg. zinc per g. dry weight, respectively, and it was, therefore, unlikely that there was a concentration of zinc exterior to the gill tissues.

Tests were then made with a radioisotope of zinc, ^{65}Zn, to trace the distribution within the body of the fish of zinc taken up from solution. Two fish were immersed until they died in borehole water containing zinc sulphate labelled with sufficient ^{65}Zn to give a convenient level of activity; the total concentration of zinc was 20 p.p.m. Some mucus on the surface of the fish was precipitated, but no coagulated mucus was seen on the gills. The dead fish were thoroughly rinsed in distilled water to remove all traces of the zinc solution before they were partially dissected and assayed for radioactivity. The distribution of the zinc taken up by the fish during the experiment is given in Table 2. Although the greatest concentration of zinc found in the tissues was in the gills, the proportions of zinc found here were only 17 and 35% of the total amounts found in the two fish, and a considerable proportion of the zinc had been distributed throughout the remainder of the body tissues.

As there was a considerable proportion of zinc present in the gills of the fish, which was not attributable to a precipitated zinc–mucus–protein complex, it was suspected that the deaths might have been caused by damage to the tissues of the gills themselves. The gills of rainbow trout exposed to a range of concentrations of zinc were subjected to histological examination by Dr Gwyneth Parry. In sublethal concentrations of the metal (3 p.p.m. Zn) no deviations from the normal structure could be observed within 2 days; in moderately high concentrations (4·0–4·5 p.p.m. Zn) some swelling of the gill lamellae appeared before death; in

very high concentrations (20 p.p.m. Zn) cytological breakdown of the epithelium occurred within 2½ hr. No excessive secretion of mucus was found (Dr Gwyneth Parry, personal communication). This effect of zinc on gill tissues is basically similar to that reported by Schweiger (1957) for the action of nickel, mercury, and cadmium salts on the gill tissues of carp.

TABLE 2. *Distribution of zinc taken up by two rainbow trout killed in a solution of zinc sulphate containing ^{65}Zn as a tracer*

	μg. zinc per g. wet weight tissue	
Sample	Fish 1	Fish 2
Whole fish	7·35	11·6
Gills	63·2	59·6
Gut	0·9	} 1·7
Liver	2·1	
Precipitated mucus from post-anal region	—	128
Post-anal region after removal of mucus	—	13·8
Remainder	5·4	7·25

DISCUSSION

Of the variables investigated, the most important in influencing the toxic effect of zinc to fish appears to be the hardness of the water. Since the forms of the curves obtained at different hardness levels differ appreciably, there is no constant ratio between the toxicities of zinc at two specific hardness values. The effect of hardness increases with an increase in the periods of survival, until there is almost a 10-fold difference in the concentrations of zinc toxic in 2½ days in a water of total hardness 320 p.p.m. as $CaCO_3$ and a water of total hardness 12 p.p.m. as $CaCO_3$. It is important, therefore, to know the total hardness of the dilution water when comparing data on the toxicity of zinc to fish. Cairns & Scheier (1957) found that, in experiments of 96 hr. duration, zinc was four times as toxic to the common bluegill in a 'soft' water (total hardness approximately 50 p.p.m. as $CaCO_3$) as in a 'hard' water (total hardness approximately 200 p.p.m. as $CaCO_3$) which agrees reasonably with what might be expected from the curves shown in Fig. 1. Concentrations of zinc found in this work to be toxic in a given time to rainbow trout in a hard water (total hardness 320 p.p.m. as $CaCO_3$) are slightly lower than those given by Goodman (1951) for rainbow trout in a 'hard' water (hardness unspecified); one possible explanation is that as the water supplied to his fish stock tanks contained about 1 p.p.m. zinc, it is probable that acclimatization to zinc poisoning had occurred at this sublethal concentration. Figures for toxic concentrations of zinc to fish based on experiments made in either distilled water, or water of very low mineral content in which control fish died, are of little practical value.

Although Jones (1938) explained the protective action of calcium salts by showing that the presence of calcium chloride prevented the precipitation of mucus by lead salts, this cannot provide an explanation of the role of calcium described here, because fish were killed in solutions of zinc salts without any coagulation of mucus

on the gill filaments. Similarly, the reduction in toxicity cannot be due to precipitation of zinc from alkaline solutions, as, at the pH values employed, the concentrations used in most of the tests did not exceed the solubility of zinc; moreover, it was shown that the precipitated zinc compounds in suspension were as toxic as a similar concentration of zinc in solution.

The concentration of dissolved oxygen is an important variable to be taken into account when assessing the toxicity of a polluted river water to fish, for it is well known that a decrease in the dissolved oxygen concentration increases the toxicity of many poisons. However, a fish will not encounter an instantaneous decrease in dissolved oxygen concentration and it will usually have some time in which to become acclimatized to a lower oxygen level. It has been shown here that the sensitivity of rainbow trout to solutions of zinc salts in water of low oxygen concentration is reduced if the fish are first acclimatized to the oxygen level used in the test. When rainbow trout are not acclimatized to the lower oxygen concentrations, the ratio of the concentrations of zinc required to give a median period of survival of 1000 min. in water containing 100% and 40% of the air-saturation value of oxygen is 1 : 0·7; this is probably the maximum effect that can be caused by such a decrease in the oxygen concentration of the water.

It is generally accepted that an increase in temperature of 10° C. reduces the median period of survival of fish in toxic solutions by a factor of between 2 and 3. In the present experiments the calculated factor for the decrease in the median periods of survival caused by an increase in temperature from 12·0 to 22·0° C. was 2·35. However, there is no evidence that the relation between the logarithm of the median period of survival of rainbow trout in a given concentration of zinc and temperature is linear over the whole range of sublethal temperatures, so that this factor may not apply to differences of 10° C. at lower temperatures. Temperature influences the time of survival only, and the threshold concentration of zinc appears to be similar for all temperatures tested.

Since, in most of these experiments, precipitated mucus was not observed on the gills of the rainbow trout killed in solutions of zinc salts, the cause of death could not be attributed to this phenomenon. It was found in the present experiments that the greatest concentration of zinc in the tissues of fish killed in a solution of zinc salts was in the gills; similar results have been obtained by Saiki & Mori (1955). However, these authors found that if fish were injected with solutions of zinc salts, the greatest concentration of zinc found in the body after 45 hr. was in the kidneys, with only a small proportion in the gills. Schweiger (1957) analysed the tissues of carp killed in solutions of cadmium or manganese for the concentrations of these metals in the fish, and then injected carp with three times the amount of cadmium or three hundred times the amount of manganese found there. These fish survived for at least 3 months. Thus there is some evidence that the heavy metals are not general internal poisons but that they act specifically on the gills of fish. The histological examinations of Parry (reported here) and Schweiger show that the gills of fish killed in solutions of heavy metals are severely damaged, but the precise action of the heavy metals in causing such a disruption is still not known.

The author wishes to thank Dr Gwyneth Parry of the Ministry of Agriculture, Fisheries and Food for determining the freezing-point depressions of the blood of fish kept in the artificially prepared soft waters.

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THE EFFECT ON WOOD-PIGEON BREEDING OF SYSTEMATIC NEST DESTRUCTION

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(With 1 Text-figure)

Wood-pigeon nests were destroyed in various localities scattered throughout East Anglia during the breeding season of 1956. Nests were destroyed at three periods: 23 July–7 August; 14–30 September and 29 October–12 November.

Four nesting habitats were studied—hedgerow, deciduous thicket, mature deciduous woodland and coniferous woodland—amounting to approximately 100 acres of each habitat.

The density of occupied nests varied between different habitats. Hedgerow supported the highest number per acre, deciduous woodland the lowest. The average time required to clear 1 acre of occupied nests during the three trials was 2·7, 2·2, 1·4 hr., respectively. During the first two trials the average expenditure per occupied nest was 0·4 man-hours; for the third period it was 3·2 man-hours.

In 1957 nests were destroyed at two separate periods in sites in various counties in East Anglia. Control sites where no nests were destroyed were examined at fortnightly intervals throughout the breeding season and of the total number of young expected to leave their nests in the experimental sites only 37 % did so. Nest destruction prevented the other 63 % from being fledged.

Introduction

In 1953 Mr A. Wylie, then the Pests Officer for Cambridgeshire, noted the high concentration of wood-pigeon nests in the hedgerows and shelter belts that were often designed to provide cover for game. In organized trials he demonstrated the ease with which these nests could be found and destroyed, so preventing large numbers of young from being reared. Alexander (1939–40) gives details of a campaign undertaken by Viscount Reidhaven in 1879, who, on an estate in Banffshire, in 4 years from 1876 to 1879 supervised the destruction of 15,194 eggs and 1003 young. Alexander summarized opinion on nest destruction as it was in 1953. 'Its (the wood-pigeon's) breeding season is probably more extended than that of any other British bird, normally lasting from April to August or September; nests are sometimes found in March and October, and occasionally even in January and February. The nest of the wood-pigeon being a mere flimsy platform of sticks, it is difficult to distinguish new ones from those of previous seasons, and in many woods almost every tree contains pigeons' nests. The two eggs hatch after 15 or 16 days' incubation, and the young remain in the nest for about 3 weeks. Thus to make sure that no young wood-pigeons were reared in a wood, it would be necessary to destroy all pigeons' nests at intervals of about 5 weeks for a period of 5 or 6 months.'

Such a method presented many drawbacks. It was well known that if a bird was robbed of eggs or young she would in many cases repeat her nesting attempts and produce offspring. Colquhoun (1951) also showed that wood-pigeons had a long breeding season, lasting from March to October, and that of the eggs laid throughout this period about 80% failed to produce flying young. This seemed to indicate that

TABLE 1. *Percentage of wood-pigeons fledged in half-month periods throughout the breeding season*

Half-month period		% fledged 1955		% fledged 1957	
April	1	—			
	2	—		0	
May	1	—		0·2	
	2	—		—	
June	1	1·0		1·2	
	2	—		—	
July	1	1·4		1·3	
	2	2·7		4·4	
August	1	14·4		9·3	
	2	24·4		17·9	
September	1	13·7	72·1	24·1	67·3
	2	19·6		16·0	
October	1	17·5		12·9	
	2	5·2		7·2	
November	1	0		3·4	
	2	—		0	
Total no. young fledged		291		526	

The figures for 1955 are derived from data obtained in Cambridgeshire. The 1957 results represent the summation of data from various localities in the following counties: Cambridgeshire, Lincolnshire, Norfolk, Suffolk, Cheshire, Lancashire and Shropshire.

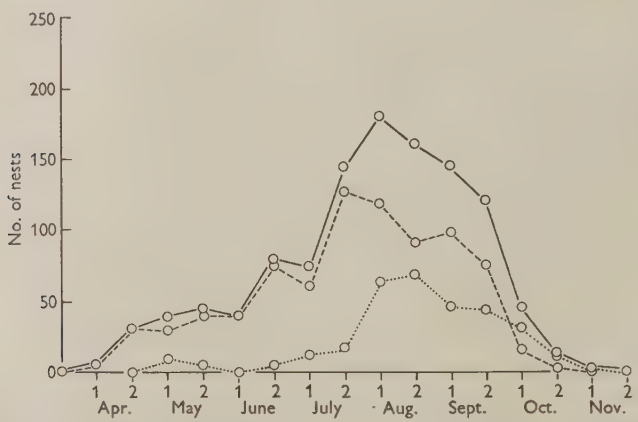

Fig. 1. Breeding season of the wood-pigeon based on the number of occupied nests found in the control sites in East Anglia in 1957. Each month has been arbitrarily divided into two halves. ○– – –○, Number of nests with eggs; ○ · · ·○, number of nests with young; ○—○, total number of nests occupied.

any attempt to prevent the rearing of young was likely to be economically impossible, as it would require too much labour for too long a time.

For a proper assessment, however, more information was needed and so an intensive study of wood-pigeon breeding biology was started in 1955 with the assistance of some members of the Cambridgeshire Pests Department, and extended in 1957 to seven counties in England. The survey (Murton, 1958) demonstrated that most birds do not start breeding until late July. This is shown in Fig. 1 where data obtained in the 1957 control sites listed in Table 6 (see below) have been used. Apart from there being more nesting birds in August and September, seasonal variations in percentage hatching and nestling success (i.e. % of young which leave their nests) result in proportionately more young being produced at this time. As a result of these three factors, 70% of all the young produced in a year leave their nests in August and September (see Table 1).

The nest and nesting habitat

A wood-pigeon's nest consists of a flat platform of twigs and sticks, graduated in size so that the lining is made of fine twigs, pine needles or sometimes grass stems. Nests used more than once can become fairly bulky but sometimes the structure is so flimsy that the normal clutch of two eggs can be seen through the bottom. The nest usually cannot be confused with that of any other species. This fact is important, as during the experiments nests were identified without reference to their contents or to the adults and it was desirable that nests of other species were not harmed. Occasionally other species utilize old pigeon nests, most noticeably, long-eared owl (*Asio otus*), tawny owl (*Strix aluco*) and to a smaller extent kestrel (*Falco tinnunculus*) and sparrow hawk (*Accipiter nisus*). By July, these species, except for the sparrow hawk, have usually finished nesting. Stock doves (*Columba oenas*) often use a wood-pigeon's nest but the species is not protected by law. The turtle dove (*Streptopelia turtur*) builds a similar nest to that of the wood-pigeon but it is much smaller and flimsier. The eggs are also considerably smaller. If there was any doubt about the identity of a nest it was not destroyed, and there was no instance of any other species being disturbed as a result of the experiments.

The nest site of the wood-pigeon varies according to the vegetation, and the maximum cover is utilized. Most nests are built on a horizontal branch, or in a fork of the main trunk. Colquhoun (1951) has produced an analysis of nest site preferences. In general the nest is easy to see owing to its size. The height at which it is built varies with the cover and this factor was of importance when destroying nests. Most nests in hedgerows and deciduous thickets were found about 10–15 ft. above the ground: those in deciduous woodland were sometimes higher, reaching 40 ft. in mature trees. The majority of nests were found at 20–30 ft., or lower in scrub undergrowth. In coniferous woodland, plantations with trees about 30–35 ft. in height were preferred, most nests being built at between 20 and 30 ft. During the trials it was quite easy to reach all nests with poles that could be extended to 40 ft.

Experiments in 1956

In 1956, nest destruction trials were carried out in selected sites distributed throughout East Anglia to determine the man-hours required to destroy the nests in each site. The four preferred nesting sites were selected for study, namely: hedgerow, deciduous thicket without high trees, mature deciduous woodland with or without a shrub undergrowth, and coniferous woodland. 100 acres of each habitat were chosen, the samples being split into small sites distributed throughout the East Anglian counties (Table 2). The original sample size was not maintained at the third trial because some occupiers refused access to their property at a time when their pheasants were in the woods. It was impossible to find comparable sites in each county, so that for a given habitat there was some variation between sites, but

Table 2. *Total size and distribution of the sites used for the* 1956 *nest destruction trials*

	Total acreage of sites in each county for the four habitats studied (no. of sites in brackets)			
County	Hedgerow	Deciduous thicket	Deciduous woodland	Coniferous woodland
Bedfordshire	5 (3)	5 (1)	5 (1)	5 (2)
Cambridgeshire	5 (5)	5 (2)	5 (1)	5 (1)
Essex	15 (13)	15 (2)	15 (1)	15 (3)
Hertfordshire	7·5 (15)	7·5 (1)	7·5 (2)	7·5 (1)
Huntingdonshire	5 (4)	5 (1)	5 (1)	5 (1)
Isle of Ely	2·5 (5)	2·5 (1)	2·5 (1)	0
Lincolnshire (Holland)	5 (21)	5 (5)	0	0
Lincolnshire (Lindsey)	20 (1)	19 (1)	20 (1)	20 (1)
Norfolk	20 (9)	20 (7)	20 (8)	20 (7)
Suffolk (East)	10 (3)	10 (2)	10 (3)	10 (3)
Suffolk (West)	7·5 (3)	7·5 (2)	7·5 (1)	7·5 (1)
Total	102·5 (82)	101·5 (25)	97·5 (20)	95 (20)

the broad habitat divisions remained well defined. The cover provided by a habitat is probably of primary importance in determining where a nest is built, and the samples chosen gave four different physical characteristics rather than four vegetation types.

Nests were destroyed during each of three separate periods. From Table 1 it is seen that not many young leave the nest until the end of July and so the first trial was undertaken during the period 23 July–7 August. The timing of the second visit depended on several factors. If adult wood-pigeons lose their nest and eggs they immediately attempt to nest again. This happens frequently under natural conditions because many eggs are taken by predators. At any one time many stages of breeding can be observed; some birds will be incubating eggs, others will have young, while a few will be building nests or be in various stages leading up to egg laying. The birds hold territories and these usually contain several nests remaining from previous years. Any of these may be selected for repeat layings or a new nest may be built. The time taken to repeat varies, depending on the physiological stage reached by the bird in the breeding cycle. Thus a bird that is ready to lay eggs, on

losing her nest, can lay them in a new nest, as quickly as one becomes available; whereas a pair feeding young have to undergo a series of changes both in behaviour and physiological condition before eggs can be laid. As a result of these factors, if all the nests in a breeding area are destroyed, repeat clutches are not laid simultaneously through the population. The eggs require 17½ days incubation before they hatch, after which the nestlings remain in the nest for a period varying between 20 and 35 days. It was calculated that a period of about 6 weeks would elapse before the sites would again contain any young approaching a free flying condition. Consequently the sites were revisited during the period 14–30 September and finally, after a similar interval, between 29 October and 12 November.

During each trial the sites were systematically searched for nests and each one found was destroyed. To facilitate this work jointed aluminium 'squirrel poles' (Shorten, 1954) were used, each having an S-shaped piece of metal fixed in a horizontal plane to the end of the pole; they served both to hook the nests downwards or to poke them upwards. Empty nests were also destroyed because, as previously mentioned, they might otherwise have been used for repeat laying attempts; moreover, from the ground, it was not always possible to tell whether a nest was empty or not.

The 1956 trials showed that the density of occupied nests varied between the different habitats and that this could be related to the amount of cover available. We had no knowledge of the sites beforehand so that places with exceptionally high or low densities were not purposely selected. Table 3 shows that hedgerows supported the highest breeding density per acre and mature deciduous woodland the lowest. The number of empty nests found in these habitats is also given. The variation in the proportion of empty to occupied nests found on the first visit depended to a large extent on the number left over from previous years. This number was highest in coniferous woodland because of the protection afforded by the dense vegetation. The proportion of occupied to empty nests was similar in each habitat on subsequent visits. The percentage occupied on the last visit was very low and this period coincided with the natural end of the breeding season.

The proportion of occupied nests containing either eggs or young was about the same for each habitat for the same period (Table 3). However, it varied between periods, an increasing proportion of young being found on visits following the first. Under natural conditions the proportion of young present in any site increases over this period (Fig. 1) and, following interference, the population assumed the structure typical for the time of year. Such a situation would be expected as predation and hence hatching success and also nestling success vary throughout the year and they are governed by outside environmental conditions which were not affected by the experiments.

As the nests were destroyed a record was made of their contents and the total time required per site was noted (Table 4). From this was obtained the average time in man-hours required to destroy one occupied nest and this did not vary much between different habitats. The longest time, 0·5 man-hours per occupied nest, was needed in deciduous woodland as here the nest density was low. On the

other hand, although nest density was highest in hedgerow, nearly as much time was required per nest, as searching was made difficult by the continual need to peer into the dark hedge. When a nest was located some manoeuvring was then usually necessary to reach it. By comparison, more nests could be found and dealt with in a proportionately shorter time in deciduous thicket and coniferous woodland.

TABLE 3. *Density of occupied wood-pigeon nests per acre in different habitats and the percentage containing eggs or young for three separate periods of nest destruction during the* 1956 *breeding season*

Habitat	Acreage	No. of nests		% occupied	% occupied nests with		Density of occupied nests per acre
		Occupied	Empty		Eggs	Young	
		First nest-destruction period					
Hedgerow	102·5	1189	3093	27·8	76·3	23·7	11·6
Coniferous woodland	95	624	2307	21·3	77·2	22·8	6·6
Deciduous thicket	101·5	682	1813	27·3	77·0	23·0	6·7
Deciduous woodland	97·5	309	935	24·8	72·8	27·2	3·2
		Second nest-destruction period					
Hedgerow	102·5	946	1498	38·7	66·1	33·9	9·2
Coniferous woodland	95	540	949	36·3	58·9	41·1	5·7
Deciduous thicket	101·5	548	998	35·4	66·2	33·8	5·4
Deciduous woodland	97·5	273	489	35·8	62·6	37·4	2·8
		Third nest-destruction period					
Hedgerow	98·5	44	537	7·6	15·9	84·1	0·4
Coniferous woodland	47	49	174	22·0	4·1	95·9	1·0
Deciduous thicket	98·5	39	317	11·0	56·4	43·6	0·4
Deciduous woodland	94·5	19	211	8·3	36·8	63·2	0·2

The average number of man-hours required to clear nests from 1 acre of habitat varied directly in proportion to the number of nests. For the first period, when many empty nests were present, the average time required for all habitats was high (2·7 man-hours) but it was successively lower for the second and third trials, when fewer nests were present (2·2 and 1·4 man-hours, respectively). Similarly, a longer time was required to clear 1 acre with a high nest density—hedgerow (4·6 man-hours)—compared with a habitat with few nests—deciduous woodland (2·2 man-hours). On a conifer site in Cambridgeshire, where nest destruction was previously undertaken in 1955, there were fewer old nests to destroy and at this site 60% of the nests were occupied at the first destruction, compared with an average of only 21% for all other sites (maximum of these others was 31%).

In 1956 no effort was made to take records at the different sites between nest

destructions so that it was not possible to estimate the success of the operations in terms of the percentage of young prevented from fledging successfully. There was some indication that the interval between periods was too long and that some birds managed to rear young between visits.

TABLE 4. *Man-hours required to destroy wood-pigeon nests per acre and per nest in* 1956

Habitat	No. of occupied nests	Acreage	Total man-hours	Man hours per acre	Total man-hours per nest
	First nest-destruction period				
Hedgerow	1189	102·5	467	4·6	0·4
Deciduous thicket	682	101·5	216	2·1	0·3
Deciduous woodland	309	97·5	169·75	1·7	0·5
Coniferous woodland	624	95·0	212·25	2·2	0·3
Total	2804	396·5	1065·0	2·7	0·4
	Second nest-destruction period				
Hedgerow	946	102·5	361·25	3·5	0·4
Deciduous thicket	548	101·5	178·25	1·8	0·3
Deciduous woodland	273	97·5	150	1·5	0·5
Coniferous woodland	540	95·0	198	2·1	0·4
Total	2307	396·5	887·5	2·2	0·4
	Third nest-destruction period				
Hedgerow	44	98·5	210·5	2·1	4·8
Deciduous thicket	39	98·5	105	1·1	2·7
Deciduous woodland	19	94·5	100	1·1	5·3
Coniferous woodland	49	47·0	67	1·4	1·4
Total	151	338·5	482·5	1·4	3·2

EXPERIMENTS IN 1957

In 1957 further experiments were designed, to measure the effect of two nest destruction operations. The number of young reared in sites where nests were destroyed were compared with the number reared in control sites, in which no nests were disturbed. Sites were selected as available in the various counties of East Anglia. Three habitats were chosen for study—hedgerow, deciduous woodland and coniferous woodland—and a sufficient number of comparable sites were obtained to provide experimental and control samples of about the same acreages (Table 5). The control sites were examined at fortnightly intervals from the start of the breeding season. Each nest was marked with a numbered metal tag and all the changes in their contents throughout the season were noted, i.e. clutch size, time of hatching, period when young were fledged, loss of eggs or young by predation or other causes. A mirror on a telescopic pole was used to facilitate nest examination. Data on the percentage of eggs hatched or young fledged and information regarding predation and other biological effects have already been published (Murton, 1958).

In the experimental sites a similar procedure was adopted, but examinations were not started until July. These sites were visited and the nests tagged and recorded between 1 and 5 July and again between 15 and 19 July. As before, any changes were noted and especially the number of young fledged. After another fortnight

the nests were destroyed between 29 July and 2 August. The same procedure was then repeated at fortnightly intervals. Two more nest inspections were undertaken between 12 and 16 August and 26 and 30 August, followed by a second nest destruction operation between 9 and 13 September. Finally, two more inspections were made, on 23–27 September and 7–11 October. By comparing the control and the experimental sites it was possible to see what happened to a wood-pigeon population

TABLE 5. *Distribution and acreage of experimental nest-destruction sites and control sites in* 1957

	Control sites			Nest-destruction sites		
Habitat	County	Acreage	Total	County	Acreage	Total
Hedgerow	Suffolk	6·6	11·0	Isle of Ely	4·9	9·8
	Norfolk	4·4		Lincolnshire (Holland)	4·9	
Deciduous woodland	Norfolk	15·4	21·5	Essex	20·0	20·0
	Suffolk	6·1				
Coniferous woodland	Cambridgeshire	2·0	9·0	Suffolk (west)	10·0	10·0
	Lincolnshire	5·0				
	Norfolk	2·0				
Total			41·5			39·8

when nests were destroyed and how many young left their nests in spite of nest destruction. The results are given in Table 6. As shown in Fig. 1, the number of occupied nests increased between July and the first destruction operation, as this was the period just before peak breeding activity was reached. After the first destruction it appeared that a few birds which had produced young ceased nesting activities, with the result that a gradual decline in the number of occupied nests was apparent before the end of the breeding season. The large proportion of empty nests found during the second destruction operation indicated the high predation suffered by these nesting wood-pigeons, the birds frequently building new nests following the loss of a clutch or brood.

The number of occupied nests in the control sites gradually increased, reaching a peak in early August (Fig. 1). In Table 7 the number of young fledged in the control sites is given and a comparison made with the situation in the experimental sites. Unfortunately, it was found that the breeding density was much higher in the nest-destruction sample than in the control areas. This was mainly because the hedgerows in Lincolnshire and the Isle of Ely contained many more occupied nests than the hedgerows in Suffolk and Norfolk, a factor which could not have been foreseen. The ratios of the sizes of the two populations are shown in the table. On average, the experimental sites supported 2·5 times the breeding density of the controls. With this information, it is possible to arrive at an approximation for the number of young which should have left the experimental sites had nest destruction not been done.

A small number of nestlings were fledged early in the breeding season and in the control group 3·3% did so before examinations of the experimental sites were

started. In the experimental group the number of young which should have fledged can be estimated as follows: 363 young were successfully fledged by the control populations; therefore $363 \times 2{\cdot}5 = 907{\cdot}5$ is the expected number which should be produced by a population 2·5 times as large; 3·3% of this total, or 29·9, would have left the nest before examinations started. At the end of the inspections there were sixty-five young still in nests. Not all these young would have flown and, in fact, it has been shown that the nestling success (the percentage of nestlings present which leave their nests) in October is 67% so that of the sixty-five nestlings left, about forty-four would have flown successfully.

TABLE 6. *The numbers of occupied and empty wood-pigeon nests present and destroyed in the experimental sites in* 1957

	No. of occupied nests	% of occupied nests with		No. of young fledged since previous visit	No. of young vanished since previous visit (not fledged)	No. of empty nests	Ratio of occupied/ empty nests
		Eggs	Young				
1st examination 1–5 July	429	76·7	23·3	Not known	Not known	1217	1:2·8
2nd examination 15–19 July	400	77·3	22·7	113	28	1484	1:3·7
1st destruction 29 July–2 August	482	82·8	17·2	84	12	1888	1:3·9
3rd examination 12–16 August	360	100·0	0	0	0	466	1:1·3
4th examination 26–30 August	341	58·1	41·9	0	0	637	1:1·9
2nd destruction 9–13 September	355	62·5	37·5	62	77	850	1:2·4
5th examination 23–27 September	92	100·0	0	0	0	251	1:2·7
6th examination 7–11 October	52	23·1	76·9	0	0	297	1:5·7

Thus, in the experimental sites, of the expected total of 907·5 young which should have fledged the actual number doing so was 259, to which total must be added thirty (the number estimated to have been fledged before inspections started) and forty-four (the number estimated to have fledged after the experiment finished) which gives a total of 333. Nest destruction lowered the expected total of flying young from 907·5 to 333. The method therefore effectively prevented about 63% of the expected young from leaving their nests.

The timing of the trials was important; the bulk of the young which left their nests before nest destruction was started were in the Lincolnshire hedgerows (ninety-five young as against eighteen in the other sites). Of the sixty-two young which flew before the second operation fifty were in the Lincolnshire hedgerows. Had the second operation been slightly earlier many of these young would have

been killed. Again, had the first destruction been earlier, it also would have prevented many birds from leaving the nests.

TABLE 7. *A comparison of the number of young wood-pigeons fledged from the control and experimental sites in* 1957 *and the breeding density in the two groups based on the number of occupied nests*

		Control sites			Experimental sites		
Half-month period		No. of occupied nests (1)	No. young fledged	Date	No. of occupied nests (2)	No. young fledged	Ratio of the no. of occupied nests in the two samples. Columns (1):(2)
Apr.	1	5	—	—	—	—	—
	2	32	—	—	—	—	—
May	1	39	1	—	—	—	—
	2	47	5	—	—	—	—
June	1	42	6	—	—	—	—
	2	82	0	—	—	—	—
July	1	75	0	1–5 July	429		1:5·7
	2	144	14	15–19 July	400	113	1:2·8
		(163)*	—	**29 July–2 August**	**482**	84	1:3·0
Aug.	1	182	27	12–16 August	360	0	1:2·0
	2	160	71	26–30 August	341	0	1:2·1
Sept.	1	145	89	**9–13 September**	**355**	62	1:2·4
	2	120	54	23–27 September	92	—	1:0·8
Oct.	1	47	47	7–11 October	52	—	1:1·1
	2	13	31	—	—	—	—
Nov.	1	1	18	—	—	—	—
	2	0	0	—	—	—	—
							(mean)
Total			363			259	1:2·5

* This figure has been calculated as mid-way between adjoining values. The dates and figures in bold type refer to nests destroyed.

It was shown (Murton, 1958) that a breeding wood-pigeon population produces approximately enough young to effect a potential replacement of the adult numbers in one year. The maximum number of occupied nests present at any time during the breeding season is a fairly good indication of the number of breeding pairs present in a population. It is seen that in the control sites this number was 182 and, if it represents the approximate number of pairs, 364 birds produced 363 young during the breeding season. Applying the same method to the experimental sites the maximum breeding density was 482 pairs, and, therefore, 964 young should have been produced. The estimated total of 907 by the first method is therefore probably low, because the total of 259 young actually fledged was under artificial conditions, two disturbances having taken place. The approximation of a 63% success for the trial is therefore probably a conservative estimate.

If three operations had been conducted instead of two, considerably more young could have been prevented from leaving their nests and an estimated 80% success could have been achieved.

The cost of nest destruction work

It is well known that in wild animals mortality of juveniles is usually much higher than that of the adults. Thus animals produce far more offspring than are required to maintain the adult populations at a stable level, the surplus offspring being killed by natural regulatory agents. This situation holds for birds and Lack (1954) has summarized the available data. Any control measure aimed at the juvenile stages of an animal's life history is liable to be wasteful because a high proportion of the young killed would inevitably die through natural causes. The value of this method can therefore best be judged by considering the cost of removing potential adults and comparing this cost with that of established methods.

The most satisfactory way of defining cost seems to be in terms of the man-hour expenditure required to kill one potential adult and for this the adult and juvenile mortality must be known. It will be shown elsewhere (Murton, *in prep.*) that annual adult mortality is about 38%, based on the recovery of marked birds of known age. Juvenile mortality cannot be estimated by this method and an alternative technique involves counting the proportion of adults to juveniles in a population and noting the change in this ratio over a given time. The method is open to the criticism that any behaviour differences between the two age groups causes a bias in the results. However, by making some assumptions, it is possible to obtain an approximation for juvenile mortality. If in a stable population about thirty-eight out of every 100 adults die each year, then this number of new birds or juveniles are required each year to replace them. It has already been stated that 100 adults produce approximately 100 young, but of these only thirty-eight are required so that sixty-two must be lost, i.e. juvenile mortality is approximately 62% per year. (Wood-pigeons breed in their first year of life.) By using this figure it is possible to calculate the approximate cost of killing potential adults by nest destruction.

It was shown in Table 4 that the average time required to destroy wood-pigeon nests was 0·4 man-hours per nest. In 1957, 837 occupied nests were destroyed. For this, 334·8 man-hours would be required. It was shown above that approximately 575 (908 − 333) young were prevented from flying by these operations; if these 575 young had flown, about 62% would have died naturally. In other words, only about 219 were potential adults. About 1·5 man-hours were required to destroy each potential adult.

From Tables 4 and 5 it can be calculated that a total of 186·0 man-hours would be needed for destroying the nests in the 40 acres examined in 1957. On this basis the cost per potential adult is 186/219 = 0·8 man-hours. For three periods of nest destruction the man-hours per acre would be 243. However, in this case it is estimated that about 80% of the potential flying young would have been prevented from leaving the nest which is (908 − 30) × 80/100 (30 = the number flying before July), which would be about 702 birds. Of these 38% or 267 would survive to the adult stage. The cost would be 0·9 man-hours per bird. Records were kept of every nest destroyed and, although this was done by a second person, time was lost in

calling out the results from one person to another. In practice the times taken to destroy nests would be rather less than those in the experiments.

There is evidence that three periods of nest destruction would be more efficient than two and that the cost of killing potential adults would be the same as for two periods. However, the total cost to the person undertaking the work would be higher.

In considering a new method of control it is desirable to compare its efficiency and cost with methods already in use. Shooting is the established method of control at present but it is difficult to obtain reliable figures for its cost. The records kept by the Ministry of Agriculture, Fisheries and Food show that about three cartridges are used per bird. However, this figure is certainly biased, as many shooters do not like admitting to requiring more than a minimum number of cartridges per bird. Five cartridges per bird would probably be a fair estimate. At a cost of 6*d*. per cartridge it is seen that each adult costs between 1*s*. 6*d*. and 3*s*. to kill without including the shooter's time. This is often given free as wood-pigeon shooting is regarded as a sport. Shot birds also have a market value so that some of the costs can be recovered.

Nest destruction would seem to be a little more expensive than shooting, at about one man's wages per hour per potential adult. But organized nest destruction appears to be far more efficient than shooting and can be practised by relatively less skilled operators.

The trials described here necessitated a considerable amount of time and labour and could not have been carried out by any one person. The work was done by members of the Pests Departments of the Ministry of Agriculture in the East Anglian counties. It is not possible to mention everybody who took part in the investigation by name but my thanks are due to all. I should like also to thank those people who kindly allowed this work to be carried out on their various properties. Finally, Dr I. Thomas gave much helpful advice regarding the final presentation of this work for which I am grateful.

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MOVEMENT OF EELWORMS

VI. THE INFLUENCES OF SOIL TYPE, MOISTURE GRADIENTS AND HOST PLANT ROOTS ON THE MIGRATION OF THE POTATO-ROOT EELWORM *HETERODERA ROSTOCHIENSIS* WOLLENWEBER

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(With 12 Text-figures)

The optimum crumb sizes for movement of potato-root eelworm larvae in a sandy loam, a heavy clay and a peat soil were 150–250 and 250–400 μ. Mobility was very similar in clay and sandy loam, in both of which there was an optimum suction for movement. In the peat, however, mobility increased with suction and no optimum suction was established. Larvae may be able to move in peat at high suctions because friction between the larvae and the peat crumbs is less than between clay or sand crumbs. Larvae moved to the wet end of a moisture gradient in sand, the number increasing with the steepness of the gradient. The rate of spread of larvae in sand 150–250 μ diameter varied between 2 and 3 cm. a day, depending on suction. As pore size increases, any upward movement in a moisture gradient is opposed by falling under gravity. Larvae do not respond to a moisture gradient or fall under gravity in sand where the width of the pore approximates to the diameter of the larva. The presence of host roots also counteracted the response to a moisture gradient; the degree of orientation to the roots increased with the time the roots were in the sand. Direct observation on larvae, newly emerged from cysts, in the presence of host plant roots, suggests that larvae orientate themselves at a distance from the root and do not reach the root by random movement. Many of the movements of eelworms are explicable by considering the relationship between pore size, eelworm diameter and water distribution, and a diagram relates movement and various soil factors.

Potato-root eelworm has been reported as a pest in many different soil types but the correlation between soil type and the level of cyst infestation is not clear. Ahlberg (1952) claims that a higher build-up occurs in heavy clay soils, Schmidt (1955) states that light sandy soils favour increases in populations of potato-root eelworm, whilst Grainger (1951) suggests that there is no correlation between soil type and the level of cyst infestation. Wallace (1956) has stressed that the operative factor in soils influencing larval emergence from cysts is soil structure rather than soil type and it is possible that the same arguments can be applied to migration of larvae in soil. Wallace (1958*a*, *b*) has also shown that the distribution of water in soil pores influences eelworm mobility, but these experiments were conducted under conditions of constant suction and temperature and no attempt was made to determine the effect of moisture gradients on mobility. Moisture gradients are an essential feature of soil in the field, their steepness being influenced by soil structure, depth of water table, evaporation, rainfall and other factors. The influence of

plant roots on eelworm movement has received some attention (Bergman & Van Duuren, 1959; Peacock, 1959; Wallace, 1958*c*, *d*; Wieser, 1955, 1956), but the mechanism of eelworm orientation is imperfectly understood. An attempt has been made in this paper to examine the relationship between soil type and mobility and to study the behaviour of potato-root eelworm larvae in a moisture gradient and in the vicinity of host plant roots.

Methods and materials

To study mobility in different soil types a fen peat from Ely, a sandy loam from Spalding and a heavy gault clay from Cambridge were used. The soils were air-dried and sieved to give four crumb sizes: 75–150, 150–250, 250–400 and 400–800 μ. Larval mobility was determined by counting the percentage of larvae which migrated more than 2 cm. from the inoculation site at one end of a polythene tube 0·5 cm. in diameter, containing the soil at a known suction (Wallace, 1958*a*).

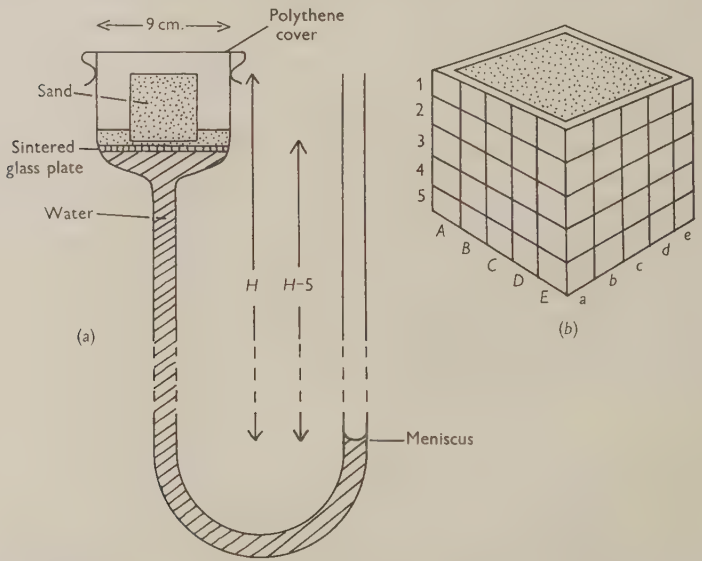

Fig. 1. Apparatus for the study of larval mobility in sand with a moisture gradient. (*a*) sintered funnel containing wax cube and sand with a suction of *H* cm. of water at the top of the cube and *H*-5 cm. at the bottom; (*b*) wax cube of 5 cm. side containing sand; the cube is marked to show how it is cut up into 125 smaller cubes of 1 cm. side.

The influence of moisture gradients was investigated by applying suction to a cube of silver sand of 150–200 μ particle size in a sintered glass funnel in a constant temperature room at 20° C. (Fig. 1*a*). The sand was contained in a hollow wax cube of 5 cm side open at both ends. The difference in suction between the top (*H*) and the bottom (*H*-5) of the cube is always 5 cm. but the difference in moisture

content depends on the part of the moisture characteristic selected for an experiment (Fig. 7). When the suction in the sand cube had been adjusted it was removed from the funnel and larvae were introduced into the centre by making a hole in the sand to the required depth, inoculating about 400 larvae into the bottom of the hole in a very small drop of water and then replacing the sand. Glass plates were then placed over the open ends of the wax cube containing the sand to prevent evaporation and the whole was placed in a box lined with polythene. After 16 hr. the cube was cut up with a razor blade into 125 1 cm. cubes (Fig. 1*b*). The larvae in each cube were then counted. This method enabled the spatial distribution in the complete cube to be determined.

Larvae used in the experiments were obtained from cysts placed in potato root diffusate in watch-glasses. The larvae were removed from the watch-glasses within a day of hatching and kept in a small drop of water for 30 min. before inoculation. This ensured that larvae were about the same age and had a uniformly high activity in each experiment.

Results

Soil type

The relative mobilities of larvae in the four crumb sizes in the sandy loam, clay and peat soils are shown in Figs. 2–4, respectively. A range of suctions was used so that mobility could be determined in relation to water distribution as indicated by the moisture characteristic. Each point on the mobility curve is the mean of two replicates. A comparison of Figs. 2–4 reveals that:

(1) For all three soil types mobility is greatest in the 150–250 and 250–400 μ crumb sizes.

(2) There is little difference in mobility between the clay and sandy soils.

(3) In all three soils mobility is very low in the 75–150 μ crumb size.

(4) The mobility curves in the 400–800 μ crumbs are similar in the three soils.

(5) The mobility curves in the 250–400 and 150–250 μ crumbs in the peat soil are different from those in the sand and clay soils. In the peat soil mobility increases as suction increases and does not show a peak as in the other two soils. Thus, the larvae have a high mobility at 400 cm. of water suction, whereas in the clay and sand mobility is very low even at 200 cm. of water suction.

Attempts to determine the factors which enable potato-root eelworm larvae to move at such high suctions in peat have not been successful. The peat soil has a higher oxygen consumption than the other soils but this might be expected to inhibit movement. Determination of pH in M/100 $CaCl_2$ with a 2:1 water:soil ratio showed that the sand, clay and peat soils had values of 5·9, 5·95 and 6·0, respectively, differences which are probably negligible. It is possible that friction between the eelworm's body and the peat crumb is less than that between the clay and sand crumbs. This would enable the larvae to overcome the restraining forces of the water films at much higher suctions in the peat soil than in the other two soil types. An experiment on the mobility of larvae in glass beads, sand and graphite of similar particle size suggests that mobility decreases as the particle surface

becomes more irregular (Fig. 5). This can be interpreted as a friction phenomenon. When the three types of particles are examined under a microscope the glass beads appear to be smooth, whereas the sand has numerous protuberances and sharp edges. Although the graphite has some flat smooth surfaces the edges are very sharp

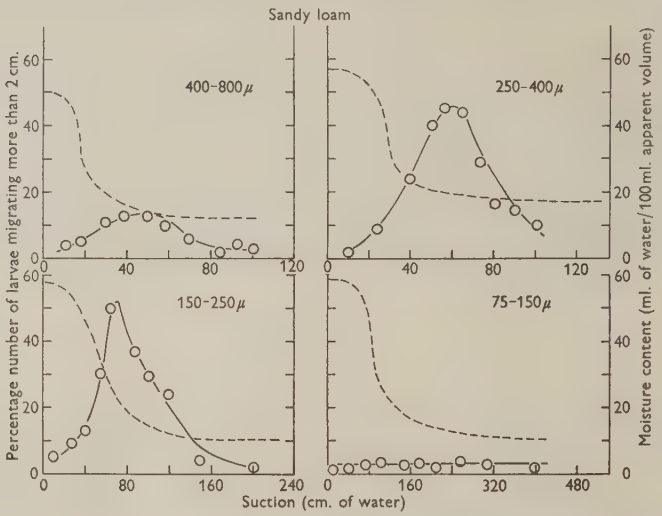

Fig. 2. Larval mobility in four crumb sizes of sandy loam: the dotted line indicates the moisture characteristic.

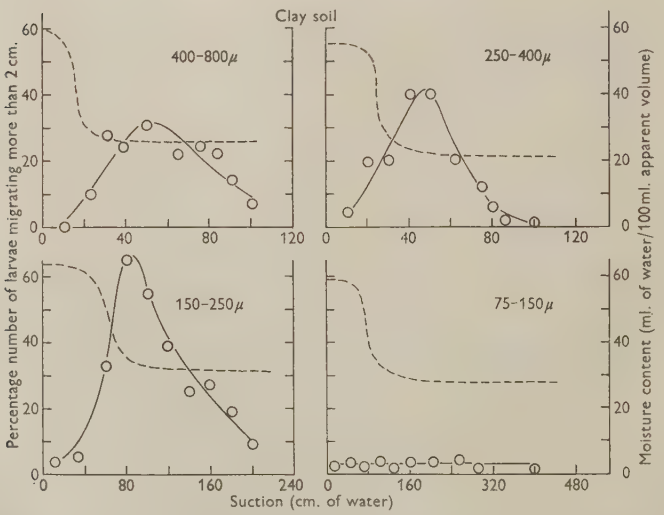

Fig. 3. Larval mobility in four crumb sizes of a clay soil: the dotted line indicates the moisture characteristic.

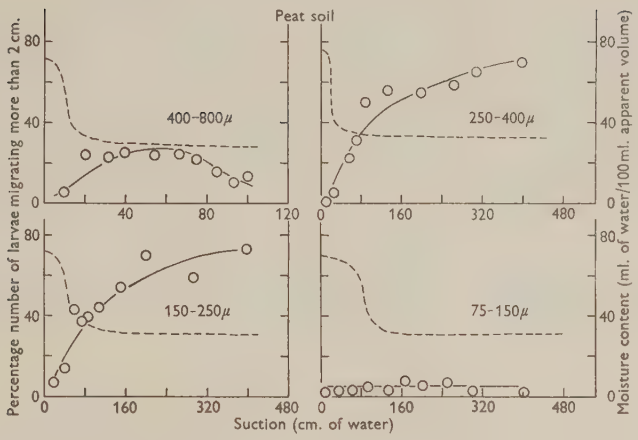

Fig. 4. Larval mobility in four crumb sizes of a peat soil: the dotted line indicates the moisture characteristic.

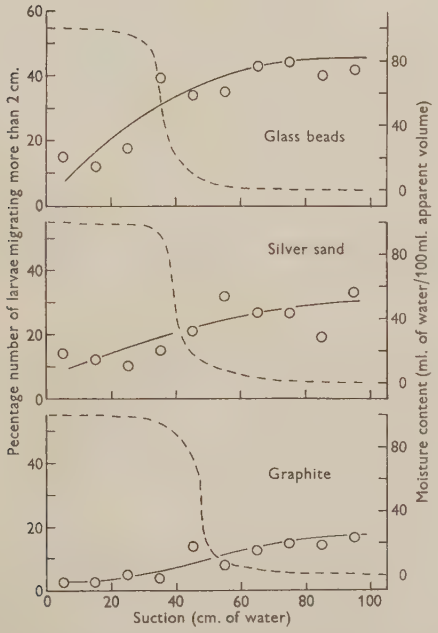
Glass beads
Silver sand
Graphite
Percentage number of larvae migrating more than 2 cm.
Moisture content (ml. of water/100 ml. apparent volume)
Suction (cm. of water)

Fig. 5. Larval mobility in glass beads, sand and graphite of the same particle size. Dotted lines indicate the moisture characteristic.

and jagged. Further research is needed, however, on the properties of peat soil in relation to eelworm mobility before any further suggestions can be made.

The fact that mobility is similar in two such widely differing soil types as sand and clay suggests that soil structure rather than the proportions of clay, silt and sand is important, with the qualification that in peat soils other factors may play an important part. The high larval mobility in fen peat may have some bearing on the fact that potato-root eelworm and beet eelworm are particularly abundant and active in the Fen district of England.

Migration in a moisture gradient

The spatial distribution of larvae in 5 cm. cubes of sand with a moisture gradient was determined. In an initial experiment a suction of 60 cm. of water was applied

(a)

	A	B	C	D	E
1	1	0	1	3	0
2	5	6	5	7	3
3	5	16	103	20	11
4	13	21	41	20	18
5	11	23	31	26	13

Wet end of moisture gradient at bottom

(b)

	A	B	C	D	E
1	19	13	15	61	25
2	20	67	140	18	18
3	9	96	224	19	6
4	0	1	0	0	1
5	0	1	5	0	0

Wet end of moisture gradient at top

Fig. 6. Vertical distribution of larvae in cubes of sand of 150–250 μ particle diameter with a moisture gradient, 16 hr. after inoculation at the centre.

measured from the top of the cube; thus, there was a suction of 55 cm. of water at the bottom of the cube. The larvae in each of the 125 cubes of 1 cm. side were counted after being in the sand for 16 hr., and from this the vertical distribution was worked out (Fig. 6*a*). The figures suggest that the larvae either move to the wet end of the moisture gradient at the lowest suction or move downwards irrespective of the gradient. A further experiment was then done with exactly the same treatment except that, before inoculating with the larvae, the cube of sand was turned upside down so that the wet end of the gradient was at the top. The moisture gradient changes little under these conditions because the water in the sand is confined to isolated pockets of water where particles touch and a flow of water cannot occur. The spatial distribution of larvae was again determined (Fig. 6*b*) and in this experiment there was a tendency for the larvae to move upwards to the wet end of the gradient. It was deduced from this that the larvae were orientating

themselves to the moisture gradient and not to gravity. Subsequent experiments were always conducted with the wet end of the gradient at the top of the block.

A series of experiments was done with different suctions to determine the amount of movement to the wet end of the gradient. The number of larvae in the top two layers at the wet end was expressed as a percentage of the total worms in the top two plus the bottom two layers, i.e. the middle layer was omitted. The moisture gradient at the different suctions depends on the form of the moisture characteristic. Thus a suction gradient of 45–50 cm. of water has a moisture gradient of 9–15%, whilst a suction gradient of 75–80 cm. of water has a moisture gradient of 5–5·3%. The results (Fig. 7) show that with increasing moisture gradient the percentage of larvae moving to the wet end increases.

The data also yield information about mobility. By calculating the distance between the mid-point of the cube and the mid-point of each of the 125 1 cm.

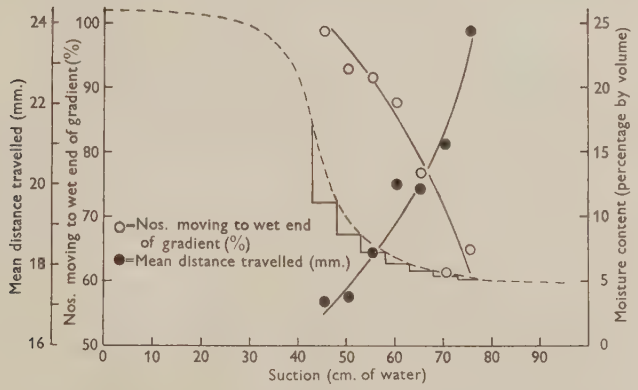

Fig. 7. Relationship between: (*a*) suction and larval mobility, and (*b*) moisture gradient and movement to the wet end. The dotted line indicates the moisture characteristic and the horizontal and vertical lines show the steepness of the moisture gradient at different suctions.

cubes (D) it was possible to tabulate numbers of worms (N) against distance travelled from the centre. For any one experiment the mean effective distance travelled by larvae in the cube is given by the sum of $N \times D$ for each cm. cube divided by the total numbers of worms in the cube (omitting those in the centre cm. cube). This may be taken as a measure of mobility or spread. When the mobility was plotted against suction (Fig. 7) mobility increased as suction increased, which agrees with previous results for beet eelworm (Wallace, 1958*a*) that in a sand of 150–250 μ particle size mobility increases with suction until a maximum is reached at a suction of about 100 cm. of water.

Under the conditions described in these experiments potato-root eelworm larvae might be expected to have a rate of spread of about 2–3 cm. a day depending on suction.

In the experiments just described, sand of one particle size, 150–250 μ, was used. The question arises whether orientation to a moisture gradient occurs in other particle sizes. To study this, suction was applied to sand of different particle sizes in horizontal polythene tubes 5 cm. long and 0·5 cm. wide to give a constant suction and moisture content throughout the length of the tube. About 100 potato-root eelworm larvae were inoculated at the mid-point of the tube through a small hole which was subsequently sealed with vaseline. The tubes were then arranged

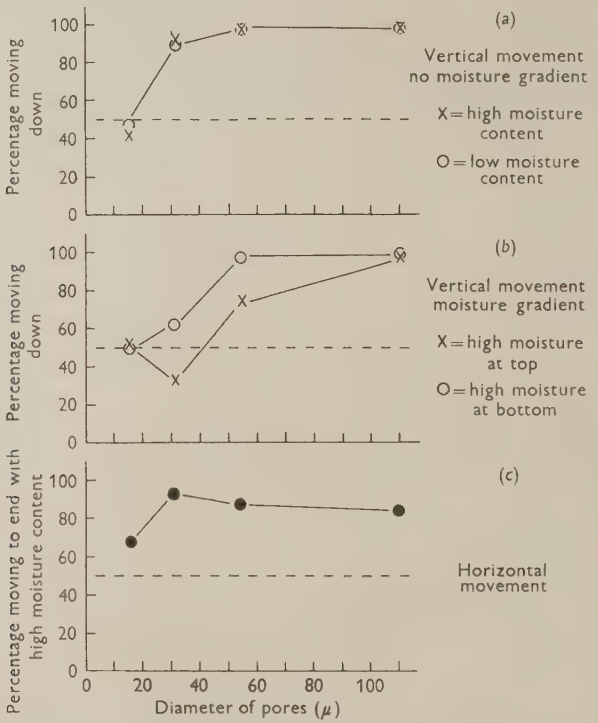

Fig. 8. Movement of larvae in pores of different size.

vertically and left for 16 hr., cut into cm. lengths and the larvae counted in each length. The number of larvae recovered from the bottom two cm. lengths was expressed as a percentage of the total number of larvae in the tube. Five replicates were used at each of four particle sizes and the results (Fig. 8*a*) were plotted as percentage of larvae moving down against mean pore diameter.* The experiment was conducted at both a high moisture content, where most of the pores were filled with water, and at a low moisture content where most of the pores were emptied of water. The results indicate that with no moisture gradient: (i) in the three sand

* By pore diameter is meant the minimum diameter across the neck of the pore.

fractions with the larger pores, almost all the larvae move downwards; (ii) in the smallest sand fraction with a mean pore diameter of 19 μ the distribution of larvae is almost 50% up and 50% down; (ii) the results are the same for pores filled with, or emptied of water.

A similar experiment was then conducted except that a suction of 60 cm. of water was applied to vertical tubes to give a moisture gradient. One set of tubes was arranged with the wet end of the moisture gradient at the top and another set with the wet end at the bottom. The results (Fig. 8*b*) show that when the wet end of the moisture gradient is at the bottom more larvae tend to move down than when it is at the top. As pore diameter decreases, fewer larvae tend to move down until in the 19 μ pores a 50–50% distribution occurs. When the wet end is at the top, (i) more larvae tend to move down in the coarser sand fractions; (ii) in the 31 μ pores, which corresponds to sand of 150–250 μ diameter used in the cube experiments, more larvae move up than down and (iii) in the smallest sand fraction a 50–50% distribution is obtained.

A third experiment on similar lines was conducted using tubes of sand with a moisture gradient but arranged horizontally. The results (Fig. 8*c*) indicate that whereas movement to the wet end of the gradient occurs in all sand fractions, it is most pronounced in the sand with pores of 31 μ diameter and least in the 19 μ pores.

These three experiments emphasize the important influence of pore size on vertical movement. With pores over about 50 μ diameter the downward movement of larvae is greater than any counter-attraction upwards to a moisture gradient. The optimal pore size for response to the moisture gradient is 31 μ which occurs in sand of 150–250 μ diameter and it has already been shown that this size of sand is the optimum for mobility (Wallace, 1958*a*). In the smallest sand fraction the larvae do not respond to gravity and it is doubtful whether they orientate themselves to a moisture gradient. In vertical tubes with no moisture gradient, downward migration is apparent in sand with pores of 31 μ diameter. It is difficult to visualize how this could occur by a simple gravity effect because the pathways for movement are relatively small and tortuous. It is possible that a similar situation exists to that described by Peters (1952) for vinegar eelworm, which collects at the surface of the liquid because the eelworms are tail-heavy and so tend to swim upwards. Observations on larvae falling in water in deep dishes show that they are head heavy, so in a sand of 31 μ pore diameter they may become orientated with heads downwards and so move downwards. This would accentuate any gravity effect.

Influence of host plant roots

It has been shown in the previous section that vertical movement of larvae may be influenced by gravity and moisture gradients. It is also known that plant roots exert an attractant stimulus to larvae (Wallace, 1958*c*; Wieser, 1955, 1956). To study the behaviour of larvae with both a moisture gradient and host roots present the sand-block method was used with twelve germinating tomato seeds included in the sand. In some experiments the seeds were placed at the wet end of the moisture

gradient and in others at the dry end. The effect on larval movement of leaving the seeds to grow for various times before inoculating with larvae was also studied. In each experiment larvae were left in the sand for 16 hr. (Fig. 9). The seeds

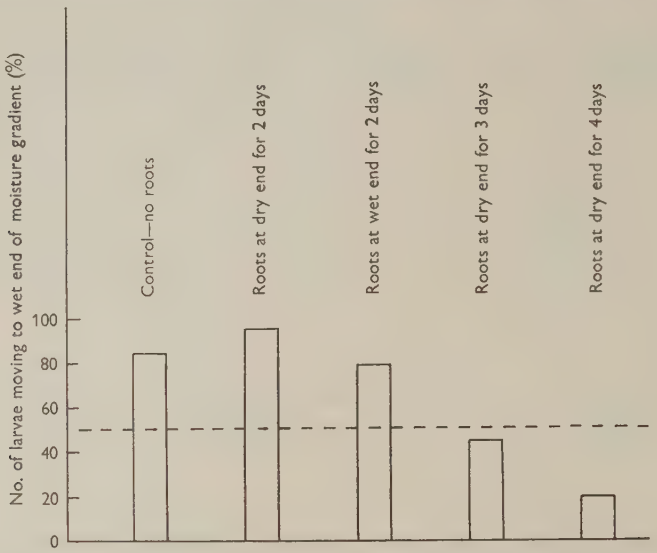

Fig. 9. Response of larvae to a moisture gradient in the presence of host plant roots. The horizontal dotted line indicates the 50 % level.

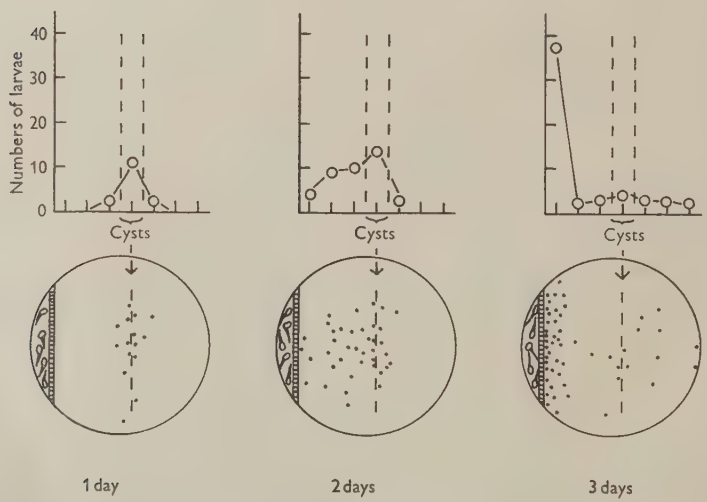

Fig. 10. The distribution of newly emerged larvae in relation to host plant roots. Each dot corresponds to one larva. The graphs above the diagrams of Petri dishes show the distribution of larvae at different times.

exerted little effect on the tendency to migrate to the wet end of the moisture gradient until they had been growing at the dry end for 3 days. The attractant properties of the roots were more pronounced after 4 days. It is concluded that there is a time lag for the larvae to respond to the presence of the roots and this tends to support previous suggestions (Wallace, 1958*c*) that the root gives out a chemical attractant which diffuses through the medium and forms a concentration gradient.

There are at least two mechanisms by which larvae can be attracted to roots. They may orientate themselves to the gradient of the attractant and so move to the roots from a distance, or the larvae may move at random and on reaching the root tend to remain there; in time this would result in an accumulation of larvae at the roots. The results with tomato seedlings in sand blocks suggest that the first hypothesis is the more probable.

It was thought that direct observation might throw some light on this problem. Twenty cysts were, therefore, placed in a line in the centre of a 2 in. Petri dish the floor of which was covered with a single layer of glass beads of 200 μ diameter. A piece of porous sintered glass was fixed at one side of the dish and five germinating tomato seeds were placed in the compartment so formed. Water was added to the dish to form a thin layer of water about the thickness of the beads. By this means emerging larvae could move under optimal conditions of pore size without being able to come into contact with the roots. Four such dishes were observed twice daily over 3 days and positions of larvae noted. The results (Fig. 10) are totals for the four dishes and show the change in distribution of the first few larvae to emerge. In the first day, emerged larvae were distributed around the cysts. During the second day there was an apparent movement to the roots and this was more definite by the third day when most of the larvae were congregated at the porous plate. There was no indication of random movement all over the dish and it is suggested that these results substantiate the hypothesis that larvae exhibit a definite orientation towards growing roots by responding to some attractant chemical exuded by the roots.

Discussion

Previous studies (Wallace 1958*a*, *b*, *d*, 1959*a*, *b*) have attempted to analyse the factors that influence movement of eelworms. A number of problems have arisen which await solution, e.g. mobility in peat and the mechanism of orientation to a moisture gradient. Chrysanthemum eelworm, *Aphelenchoides ritzema-bosi*, tends to move upwards in water films on the stems of chrysanthemums (Wallace, 1959*b*). It was suggested that such movement was a negative geotropism, but this explains nothing about the factors which bring about such a migration. It may be useful to consider whether the directional movements of chrysanthemum eelworm and potato-root eelworm are, in fact, responses to similar physical factors.

It seems worthwhile to put forward a hypothesis explaining the relationship between the eelworm, pore size and moisture content in relation to vertical movement and mobility and to survey the factors which influence movement. The solution to the problem of vertical movement may lie in the relationship between the

minimum pore diameter and eelworm's body diameter. Fig. 11 is a diagrammatic representation of an eelworm larva (in transverse section) in full and empty pores of the different sizes used in the experiments described (Fig. 8). In the largest pore of minimum diameter 110 μ the eelworm lies in a large volume of water

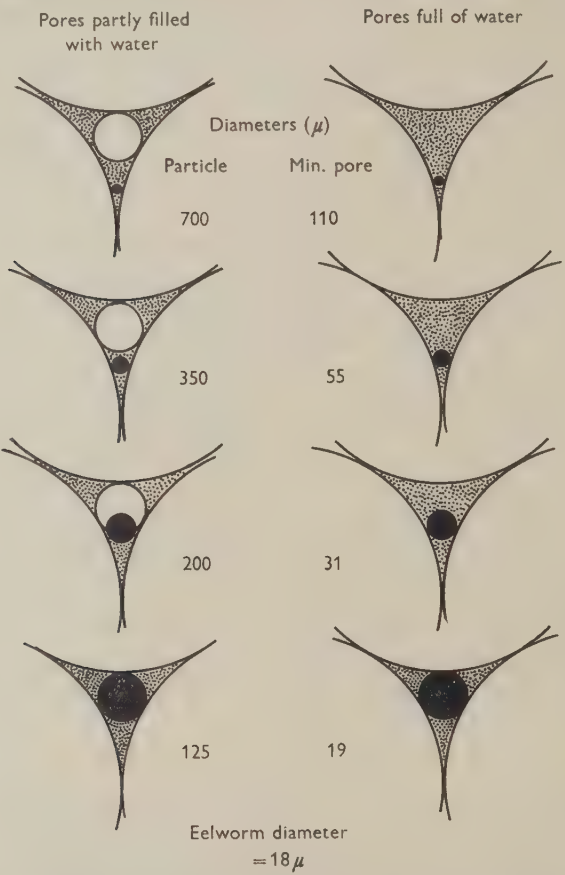

Fig. 11. Relationship of eelworm diameter to pore size and moisture content in sand. To facilitate drawing, the relative size of pore and larva is changed in each drawing. As drawn, pore size appears constant but in fact varies from 110 to 19 μ, while the larval diameter which appears to increase is constant at 18 μ. Equivalent particle sizes are also given and vary with the pore size from 700 to 125 μ. An eelworm is represented by a solid black circle and stippling indicates water.

whether the pore is empty or not and the same applies to the sand with pores of 55 μ minimum diameter. In these two sand fractions the larvae are likely to fall downwards. In sand with saturated pores of 31 μ diameter the eelworm is in a large volume of water and might be expected to fall under gravity, but where the pores are empty the eelworm's body is in contact with the water/air interface and surface

tension forces of the film tend to inhibit any downward movement. In the finest sand fraction with pores of 19 μ minimum diameter the larva can just pass through the pore, consequently the effect on them of water films is negligible so that falling under gravity does not occur. The results in Fig. 8 support this hypothesis. The degree of orientation in a moisture gradient increases as the particle size and pore size decreases until it is at a maximum in pores of 31 μ minimum diameter. To respond to the moisture gradient the larvae must be in contact with the air/water interface. This only occurs in sand of about 31 μ minimum pore diameter or about 200 μ particle diameter; with larger pores the larvae tend to fall under gravity and with smaller pores the spaces are too small for the films to have any effect.

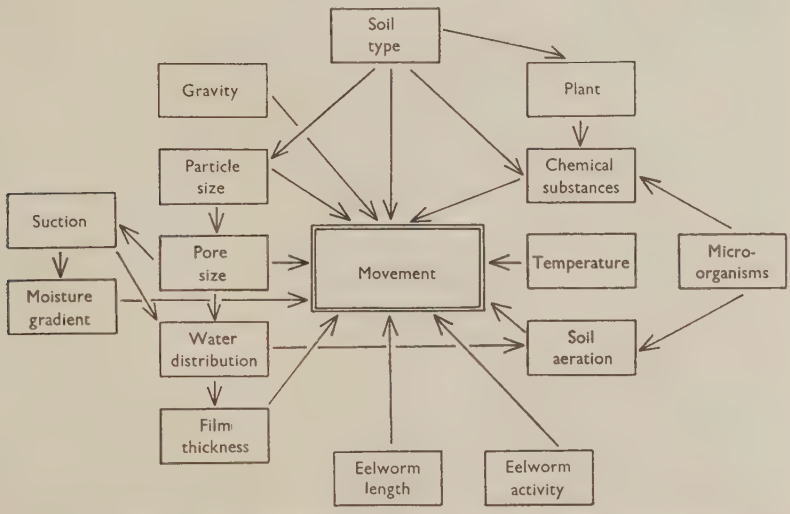

Fig. 12. Diagrammatic representation of the interrelationship of factors which influence larval movement in the soil.

The hypothesis expressed in Fig. 11 also explains satisfactorily previous results on the influence of pore size and moisture content on mobility (Wallace, 1958*a*, *b*). In sand of particle size 200 μ, the optimum for mobility, the eelworm is subjected to the surface tension forces of the water films whilst continuity of the water pathways is maintained. In larger sand fractions the air/water interface reaches the larvae only when further water has been removed from the system, thus breaking the continuity of the water films.

Many factors influence movement in soil and, moreover, these factors are often interrelated. The mobility and distances travelled by a particular species of eelworm will be determined by the environmental conditions prevailing in the soil at the time. Thus, the question of how far a particular species moves in the soil is meaningless unless the conditions are stated precisely. An idea of the environmental complex influencing eelworm movement is given in Fig 12. This diagram is similar

in many ways to that given by Wallace (1959*c*) for emergence of larvae from cysts of *Heterodera schachtii* because emergence from cysts and movement through soil pores are fundamentally the same phenomenon. The diagram probably oversimplifies the real conditions but illustrates the complexity of the situation.

I wish to thank Dr J. A. Currie for advice on some aspects of soil physics.

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THE POLLINATION REQUIREMENT OF THE FIELD BEAN (*VICIA FABA*)

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(With 1 Text-figure)

Many of the flowers on field bean plants fail to produce pods even when fully pollinated. When insect pollination is prevented pod production on the lowest inflorescences, which normally produce most pods, is reduced but a compensatory increase on the upper ones ensures a moderate yield. This change in the distribution of pods may be a useful indication of insufficient pollination.

Areas in which crops requiring pollination are grown usually contain some pollinating insects, such as honey-bees kept by local beekeepers, solitary bees and bumble bees. It is desirable that growers should be able to judge when additional bees are needed. Information useful for this purpose has now been obtained for the field bean. The investigation was undertaken because doubts had been expressed as to whether this crop was being adequately pollinated at Rothamsted.

Experiments

(*a*) *The capacity of the plant to produce mature pods when adequately pollinated*

In 1956, 200 fully open bean flowers were marked. After a fortnight about 50% had produced pods but later many of these fell off and very few remained until harvest. Thus more flowers were pollinated than produced mature pods. However, the further conclusion that pollination had been more than adequate depended on the assumption that the pods which fell off were in positions where mature pods could be produced under suitable circumstances. Pods mostly fell from the upper inflorescences and the upper flowers of the lower ones and it seemed possible that only imperfect pods could occur in these positions.

This possibility was investigated in 1957. Plants chosen at random were divided into three groups, each of which was given a distinguishing mark. Only the main stems were used for the experiment but the side shoots were not removed. The lowest four inflorescences on the stems were removed in one group and the lowest three flowers on each inflorescence in another. The third group was left untreated. At the end of the season the pods and beans on each inflorescence were counted (Table 1). Removal of flowers from the positions where mature pods are normally produced resulted in compensatory increases in production on the other parts of the plants. The removal of these flowers from a few plants in the crop could not have caused the pollinating insects to give increased attention to the remaining flowers. The increased yield of the latter shows that pollination was not the factor

which limited production in corresponding positions on the control plants and that more flowers were being pollinated than could develop to mature pods.

The pedicels remaining at each node were counted and examined and it was

TABLE 1. *Effect of flower and inflorescence removal on yield of mature pods and beans*

Treatment	Pods per plant (main stem only) Mean	S.E.	Beans per plant (main stem only) Mean	S.E.	No. of plants
Lowest four inflorescences removed	9·0	1·0	17·8	2·7	46
Lowest three flowers on each inflorescence removed	5·1	0·5	14·2	1·7	43
No flowers removed:					
All inflorescences	11·5	1·2	28·8	2·6	50
Inflorescences 5 and upwards	4·1	0·9	7·4	1·5	50
All inflorescences minus lowest three flowers on each	1·2	0·4	2·2	0·6	50

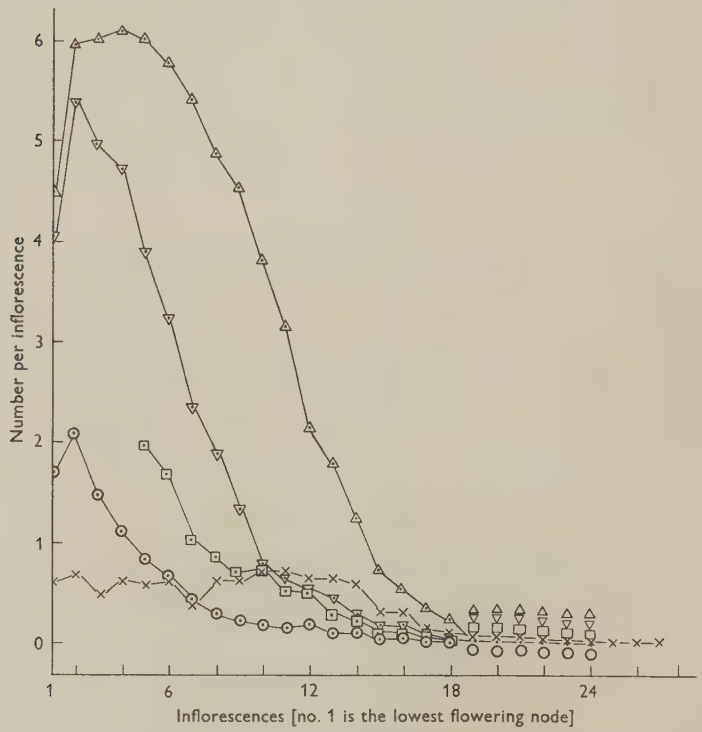

Fig. 1. Average distribution of buds, flowers and mature pods on main stem, 1957. Untreated plants: △—△, buds; ▽—▽, flowers; ⊙—⊙, mature pods. ⊡—⊡, mature pods on plants with four lowest inflorescences removed; × — ×, mature pods on plants in cage without bees.

found possible to determine roughly how many flowers had reached the bud and open flower stages (Fig. 1). On the control plants there was a diminution in the number of buds from the bottom to the top of the stems corresponding to diminution in the number of pods produced. The figures show that 17% of the buds and (less certainly) 30% of the open flowers produced mature pods. These proportions were considerably exceeded on the upper inflorescences when the lower ones were removed.

(*b*) *The performance of bean plants when insect pollination is prevented*

Nylon screen cages (9 × 9 × 6 ft. high) were used to exclude pollinating insects from plants; these were sprayed with TEPP after the cages were in position to kill any pollinating insects present. When honey-bees were subsequently put in, small colonies in hives with special entrances (Butler & Haigh, 1956) leading to the cages were used. The bees flew only in the cages except towards the end of the flowering period when they were allowed outside on alternate days.

TABLE 2. *Bean yields with and without insect pollination*

	No. of plots	Beans per stem*		Beans per stem on inflorescence 8 and upwards	
		Mean	S.E.	Mean	S.E.
No cage, 1957	1	17·2	—	1·5	—
Cage without bees, 1957	1	13·1	—	6·1	—
No cage, 1958	6	28·3	1·3	3·7	0·4
Cage with bees, 1958	3	17·6	1·9	3·1	0·5
Cage without bees, 1958	3	13·1	1·9	4·0	0·5

* Main stems in 1957; all stems in 1958.

A single cage without bees was put over part of a plot of beans in 1957. The average number of mature pods per main stem in the cage was 124% of the average outside, whereas the number of beans per main stem in the cage was 76% of that outside. The distribution of the pods on the stems is shown in Fig. 1. The number of beans on inflorescences 8 and upwards was four times as great in the cage as outside (Table 2) and the plants in the cage were taller than those outside.

A replicated experiment was done in 1958. The experimental area was divided into three randomized blocks each containing two plots without cages, one plot with a cage with bees, and one plot with a cage without bees. Table 2 shows that the number of beans per stem (all stems) was significantly less ($P < 0{\cdot}001$) in the cages with bees than on the uncaged plots, but not significantly greater than in the cages without bees. It is uncertain whether the poor yield in the cages with bees came from inadequacy of pollination or of light. The absence of a significant difference between the cages with and without bees is explained by the greater number of beans on the upper inflorescences of the plants in the cages without bees. The percentage of the beans on the whole stem which were on inflorescences 8

and upwards was significantly greater ($P < 0{\cdot}01$) in the cages without bees than in the cages with bees and the actual numbers of beans were also greater though not significantly so.

Discussion

Experimental plants could not produce mature pods on more than about one-third of their flowers although more than one-third were pollinated. Such a low level of fertility, therefore, does not necessarily indicate insufficient pollination. (A still lower proportion of pods to flowers was recorded by Hodgson & Blackman, 1956 and 1957, but the level of pollination was not investigated.)

The cage experiments show that, when pollination is inadequate, increased yield by the upper inflorescences partially compensates for the decrease on the lowest ones which normally carry most of the pods. The more uniform distribution of pods thus produced may be a useful indication of inadequate pollination in commercial crops. Conversely, it is probably safe to conclude that pollination has been adequate if the pods are crowded towards the bottom of the stems.

Insect pollination is necessary for a maximum crop but even when pollinating insects are completely excluded some beans are produced (presumably either parthenogenetically or by non-insect pollination) and compensation by the upper inflorescences ensures at least a moderate yield.

We wish to express our thanks to Dr J. Simpson for his advice and assistance throughout this investigation.

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TOPICAL APPLICATION OF MOBILE LIQUIDS TO INSECTS BY MEANS OF MICRO-CAPILLARY TUBES

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(With 3 Text-figures)

A method is described for topical application of mobile liquids, whether volatile or not, to insects. The principle is that of the self-filling micro-pipette, the 'pipettes' being calibrated lengths of fine, thin-walled glass capillary tube; for use a micro-capillary tube is mounted in a special holder. The doses delivered were shown to be adequately reproducible for mean doses of 0·03 μl. upwards. Precautions in use are: (1) in filling the capillary, its tip is dipped into the reservoir of liquid no more deeply than is necessary: (2) the dose is applied to the insect in such a way that the liquid is carried rapidly away from the delivery tip; and (3) pressure for expulsion of the dose from the capillary is maintained until after the delivery tip is removed from the insect.

A convenient apparatus for keeping a batch of insects under anaesthesia during their dosage is described.

Introduction

A variety of devices are used in the laboratory for topical application of liquids to insects, including micro-syringes, micro-pipettes, wire loops, air-pulse micro-drop applicators, and vibratory machines for producing streams of drops (for review of devices see Busvine, 1957). Each type of apparatus has its limitations: for example, an air-pulse micro-drop applicator (Hewlett, 1954; D.S.I.R., 1955, 1957) is suited to delivering only liquids of low volatility, and a micro-pipette to delivering only those of low viscosity. Moreover, each apparatus will deliver with adequate reproducibility only doses above a certain mean volume, though too few of the devices used for topical dosage of insects have been tested critically by determining the variability of doses nominally the same.

The method to be described is suitable for application of mobile liquids (of viscosity, say, 4 centipoises or less), whether volatile or not. It was developed to enable certain small beetles such as infest stored products to be dosed topically with insecticides in solvents more volatile than those for which an air-pulse applicator is suitable, but it can have much wider uses. The principle is that of the self-filling micro-pipette, but the 'pipettes' are merely short lengths of thin-walled glass capillary tube, without constricted tips. A tube, mounted in a special holder, is filled by dipping its tip into the liquid to be put on the insect; the tip is placed on the part of the insect to be treated, and the liquid is driven out of the tube by air pressure applied through the holder. A set of tubes, each calibrated and delivering a different volume, enables the volume applied to insects to be varied. For mean volumes down to 0·03 μl., and possibly less, the individual doses are

reproducible enough for treating insects, provided that simple precautions are taken.

Other methods of topical application were considered. Perry, Mattson & Buckner (1953) used a wire loop to deliver doses of 0·65 μl. of a DDT solution with an error of less than 5%. However, data are not available for the variation of lower doses delivered by smaller loops; surface tension may well influence the mean volume taken up, and perhaps a loop should be calibrated for every liquid used. For application of solutions in volatile solvents, devices in which the liquid passes in one direction only through a delivery tip are open to objection, because evaporation of solvent from the tip between deliveries can be expected to produce variation in both the volume of solution delivered and the concentration of solute. Indeed, data given by Barker (1957) suggest that the commonly used method of applying 1-μl. doses of acetone solutions to houseflies by means of a micrometer syringe needs critical investigation. We built a micro-burette of the type described by Kerr (1954) although, as Kerr points out, an apparatus of this kind is most suitable for delivery of mobile liquids of low volatility such as odourless kerosene. The burette worked well for treating dipterous flies with solutions in odourless kerosene (see also Hadaway, 1956); but the kerosene does not run from the burette on to beetles such as *Tribolium*, *Calandra* and *Alphitobius*, because the kerosene wets their cuticles much less readily than those of the flies.

Micro-capillary tubes

Manufacture. In making a micro-capillary tube, glass tubing of internal diameter about one-half the external is drawn out. The internal and external diameters of pieces of the drawn-out tubing are determined by examining the ends with a microscope, and a piece is selected such that a length of more than 5 mm. can be expected to take up the volume to be delivered; the external diameter should not exceed 0·5 mm. The length required is calculated, and cut to the nearest 0·2 mm. Generally the most convenient length for micro-capillary tubes delivering up to 0·15 μl. is 7–10 mm. As a specific example of dimensions, a tube 7 mm. long delivering a mean dose of 0·1 μl. would have an internal diameter of about 0·14 mm. and an external diameter of roughly 0·3 mm.

Holder. Fig. 1 shows a holder for micro-capillary tubes. It is similar in principle to the micro-pipette holder described by Benedetti-Pichler (1942), but has a window, making it possible to see whether the tube fills properly and whether the proximal end of the tube is clear of the wall around it. The capillary tube is held in a hole through a disc of rubber or soft plastic seated in the end of a Duralumin cap, which screws on to the end of a tubular Duralumin handle 14 cm. long. Within the cap and surrounding the proximal end of the capillary is a glass tube which compresses the disc. A slot milled through the cap, just behind the end wall, allows the proximal end of the capillary tube to be viewed through the glass tube. The thickness of the end wall of the cap, through which the capillary protrudes, and the external diameter of the distal end of the cap, are kept to a minimum, so that the

tip of the capillary can be viewed easily when in contact with the insect. A thin metal washer (not shown in Fig. 1), with a central hole equal in diameter (1·2 mm.) to the hole in the distal end of the cap, can with advantage be put between the rubber disc and the end of the glass tube to keep the micro-capillary tube properly aligned.

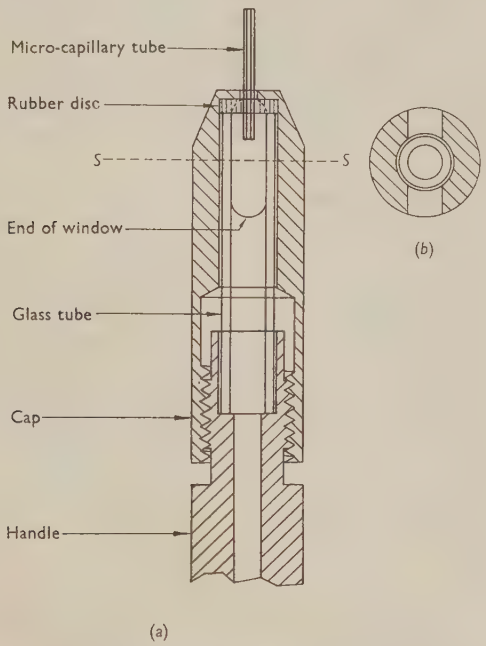

Fig. 1. The holder for the micro-capillary tubes, shown to scale. (*a*) Half-section of the end of the holder. (*b*) Transverse section in the plane *SS*. The greatest diameter of the holder is 6·3 mm.

Filling. The micro-capillary should not be dipped more deeply than necessary into the reservoir of liquid from which it is filled (see p. 130). The depth of dipping is readily controlled by inserting in the reservoir an open-ended glass tube (see Fig. 2), the contracted lower end of which prevents descent of the capillary holder below a certain level. The height of the inserted tube can be fixed by a rubber ring (Fig. 2*a*), or more easily by a threaded metal head fitting on a standard glass taper at the top of the reservoir (Fig. 2*b*).

Delivery of dose. To expel the dose of liquid from the micro-capillary tube, pressure is applied through a flexible tube attached to the proximal end of the hollow handle of the holder. The pressure can be applied by mouth, or more comfortably by means of a low-pressure air supply from a small pump. The pump is kept running continuously but, except when expelling liquid from the capillary, air

escapes to waste through a by-pass; the pressure is applied through the holder only when the by-pass is closed.

If, after emptying the capillary, air passes through it into a pool of liquid on the surface of the insect, bubbles form and burst, throwing some of the dose off the insect. Hence the liquid should be so applied to the insect as to migrate rapidly away from the delivery tip of the capillary; this happens in any case if the liquid spreads rapidly over the insect's cuticle, but, if it does not, it can be applied to the mouth of a cuticular fold, into which it will be quickly drawn (assuming, of course, a contact angle of less than 90°). Notwithstanding, it is advisable to continue pressure until the capillary is removed from the insect, to ensure that none of the dose is drawn back into the capillary.

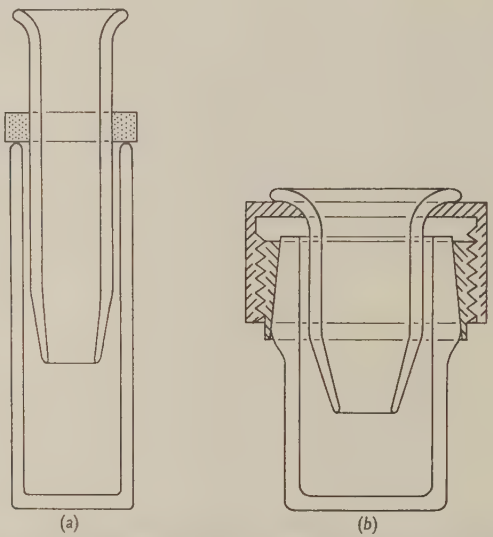

Fig. 2. Half-sections of glass liquid containers from which micro-capillaries were filled; with the height of an inserted glass tube controlled (*a*) by a rubber ring, and (*b*) by a threaded metal head.

Calibration. Each micro-capillary requires calibration, as the volume it delivers may differ from that expected, because among other things the bore of the capillary may not be uniform. The total volume of a number of deliveries, and hence the mean volume per delivery, can be estimated by a variety of methods (see text-books on micro-chemical technique, e.g. Kirk, 1950). We have used two methods.

(1) A number of deliveries of odourless kerosene are introduced from the micro-capillary into another glass capillary tube of fine, uniform, and exactly known internal diameter; and the length of the liquid column is measured with a travelling microscope. Commercially available 'precision-bore' glass tubing of internal diameter approximately 0·2 mm. is suitable, but the internal diameter of the piece to be used needs to be accurately measured (by standard methods), as the makers'

usual tolerance for the diameter ($\pm 0{\cdot}01$ mm.) is too wide (owing to its thick walls precision-bore tubing cannot be used for the topical application). The end of a short length of the precision-bore tubing is rendered oleophobic with dimethyldichlorosilane (see Anderson, 1949), a temporary plug of wax preventing contamination of the bore. After a little practice one can introduce a series of deliveries from a micro-capillary tube into the precision-bore tube, with scarcely any kerosene left on the oleophobic end. To attain the required control, the liquid is expelled from the capillary by pressure from the operator's mouth. The resulting liquid column extends along the precision-bore tube from the oleophobic end. The volume of the liquid column is given by $V = \pi r^2 (l + 2r/3)$, where r is the internal radius of the precision-bore tube and l is the distance between the proximal points of the two menisci.

(2) A set of deliveries from the micro-capillary of a densely coloured dye solution are added collectively to a known volume of a solvent, and the optical density of the resultant dye solution is estimated photo-electrically. Reference to a standard curve, giving the optical densities of appropriate dilutions of the densely coloured solution, enables the mean volume delivered by the capillary to be estimated.

For work with this method, two oil-soluble dyes, Sudan Black B and Oil Blue NA, were selected. In preparing a solution for delivery from the micro-capillaries, 10 ml. of solvent to which 0·2 g. of dye was added were shaken periodically over 24 hr. at 21–24° C. Undissolved dye was removed by centrifuging, and to reduce the concentration of dye below saturation 7·5 ml. of solution were made up to 10 ml. with further solvent. When the solutions were stored in darkness at room temperature (acetone solutions at 2° C.) their optical densities remained unchanged for at least 2 months.

TABLE 1. *Dye solutions used in photo-electric calibrations of micro-capillaries, and the volume giving an absorptiometer reading of* 0·50

Dye	Solvent	Volume (μl.)
Oil Blue NA	Odourless kerosene	6·0
Oil Blue NA	Odourless kerosene 90 %, *cyclo*hexanone 10 %*	2·0
Sudan Black B	Odourless kerosene 90 %, *cyclo*hexanone 10 %*	1·8
Sudan Black B	*Cyclo*hexanone	0·75
Sudan Black B	Acetone	0·59

* By volume.

Each set of deliveries of densely coloured solution from a micro-capillary was added to a 1-ml. volume of ethanol (for method see below), and the optical density of the resultant dye solution was measured in a 1-cm. micro-cell (capacity 0·5 ml. approx.) in a Spekker photo-electric absorptiometer. Table 1 shows the densely coloured solutions found suitable, and the volumes that gave readings of 0·50 on the absorptiometer under the conditions just indicated; these volumes will vary a little according to the exact conditions under which the densely coloured solutions are made up, and perhaps according to the sample of dye used, but Table 1 indicates the order of volumes accurately measurable. The absorptiometer was fitted with filters having maximum transmission at wavelength 610 mμ.

In order to simulate the conditions of topical application to an insect, and secure rapid migration of liquid away from the delivery tip of the micro-capillary, each set of deliveries of dye solution was made into the crevice formed by the apposed tips of a pair of forceps held closed with a clip. The tips of the forceps were then immersed in the 1 ml. of ethanol (see above), and the forceps were opened and used for mixing. When dyed acetone was delivered, the acetone evaporated, leaving a minute cake of dye that dissolved slowly in the ethanol. However, delivery of the dyed acetone just proximal to a predelivered 0·5 ml. of *cyclo*hexanone between the tips prevented the formation of a cake of dye.

Method (2) was used to investigate the effects of different factors on the volumes delivered by different micro-capillaries delivering, under the conditions of use recommended (pp. 127–8), mean volumes of up to 0·11 μl. For the liquids other than acetone (see Table 1), the volumes delivered by a given capillary were greater if the tube was dipped deeply when being filled, owing to additional liquid adhering to the external surface of the capillary. Moreover, the increase due to deep dipping was greater the more rapid the withdrawal of the capillary from the liquid reservoir. A capillary dipped deeply and withdrawn as fast as possible delivered up to 40% more than if dipped no more deeply than necessary.

Dipping the capillary deeply did not increase the volume of acetone delivered, probably owing to its low viscosity and high volatility. However, its high volatility and ability to creep over glass surfaces—properties obvious during use of dyed acetone—are drawbacks to the use of acetone as a solvent in topical dosage of insects (for objections to acetone on other grounds, see Busvine, 1951).

Flame-polishing the delivery tip of a capillary, or rendering the external curved surface oleophobic, had no significant influence on the volume delivered; nor had the rate of delivery.

When the capillaries were dipped no more deeply than necessary to fill them, the results of the calibrations were straightforward. By method (2), the mean volumes of acetone delivered were less than those of the other solvents (see Table 1), for which the means were in close agreement. The reduction for acetone ranged from about 2% at 0·11 μl. to about 5% at 0·03 μl. The mean volumes of odourless kerosene delivered were throughout estimated as 1–2% lower by method (1) than by method (2).

Variation of individual dose. In order to estimate the variation between individual doses, single deliveries from micro-capillaries were estimated radiometrically. The coefficients of variation for deliveries of an acetone solution of ^{32}P-labelled phosphate were 4·6 and 6·5% for micro-capillaries giving mean deliveries of 0·25 and 0·031 μl., respectively. Thus the reproducibility seems to be adequate for topical application of liquids to insects (for effects on dose-response data of dose-variation, see Hewlett, 1954). Using method (1) above, the coefficients of variation for single deliveries of odourless kerosene from micro-capillaries delivering mean volumes of 0·049 and 0·031 μl. were about 1%. The better reproducibility with the less volatile solvent was to be expected.

Anaesthetization apparatus

Means of immobilization of the insects during their dosage is helpful with most kinds of insects and essential with many. A special turn-table (see Fig. 3) was made to enable a batch of insects to be kept under anaesthesia for convenient dosage (for a photograph of the apparatus in use, see D.S.I.R., 1956). A table formed of a circular Duralumin plate press-fitted on to a central brass bush is mounted on a short, non-rotating, vertical brass shaft. Two concentric but separated Duralumin

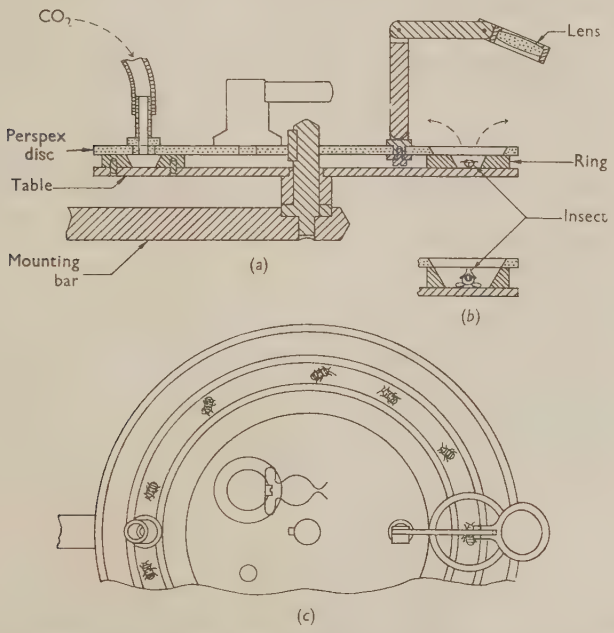

Fig. 3. The apparatus for keeping a batch of insects anaesthetized during dosage, shown to scale. (*a*) Half-section of the apparatus, fitted with a shallower pair of rings. (*b*) Detail of a half-section of the apparatus fitted with a deeper pair of rings. (*c*) Plan (apparatus fitted with shallower rings), with part cut away. The diameter of the Perspex disc is 15·2 cm.

rings rest on the table, each located by three short pins. The table and rings can be turned as one unit by a finger applied to the edge of the table, which is knurled. A Perspex disc fitting on top of the rings is keyed to the shaft and therefore does not rotate with the rings. The lower end of the vertical shaft is a press-fit in one end of the horizontal mounting bar, the other end of which is adjustably fixed, e.g. on a retort stand.

The space between the rings forms a circular channel, roofed by the Perspex disc, in which the insects are kept under anaesthesia. An anaesthetic such as

CO_2 is led through a tube located in the Perspex disc, flows round the channel, and leaves it through a hole with bevelled edges on the side of the disc opposite to the entry. Turning the table brings the insects one by one below the hole, where each is treated by means of a micro-capillary tube in its holder inserted through the hole. The insects can be viewed with the help of a lens (e.g. focal length 7·5 cm.) on a semi-universal mount fixed to the disc: if small they can be viewed with a microscope. The reservoir containing the liquid to be applied can be held in a spring clip attached to a short metal pillar located on the disc (in either a right- or left-handed position, see Fig. 3*c*).

Different pairs of Duralumin rings can be fitted to the table to suit the size of the insects to be treated. Figs. 3*a* and *c* show shallow rings fitted during treatment of insects such as the beetles *Tribolium* and *Alphitobius*. Fig. 3*b* shows a detail of a vertical section of the apparatus when fitted with deeper rings for treatment of houseflies, *Musca domestica* L.

It is convenient to have a ring of filter-paper (not shown in Fig. 3) between the rings and the table to form a floor to the channel between the rings. Glabrous insects then slip aside less readily during treatment than if on the metal table, and if dyed liquids are topically applied, accidental loss of liquid from an insect can be seen as a stain on the paper.

To operate the apparatus, the anaesthetic is set flowing slowly, e.g. at 150 c.c./min. for CO_2. The insects are introduced through the bevelled hole in the Perspex disc, the table being rotated to distribute them round the channel. When anaesthetized, the insects are arranged suitably for treatment (e.g. spaced and turned on their backs where necessary). When all have been treated, either they are removed one by one through the bevelled hole, or the Perspex disc and outer ring are removed and the insects, before recovery from anaesthesia, are swept into the container in which they will be held for observation.

Experience with the method

Micro-capillary tubes in conjunction with the anaesthetization apparatus just described have proved very satisfactory for dosage of beetles and moths of a number of species infesting stored products. These include *Tribolium* spp., *Calandra* spp., *Lasioderma serricorne* (F.), *Stegobium paniceum* (L.), and *Ephestia* spp.; beetles as small as *Rhizopertha dominica* (F.) were treated successfully. Solvents ranging in volatility from odourless kerosene to acetone have been used. *Cyclo*hexanone was found a convenient vehicle for many insecticidal compounds, for it is a good solvent, non-toxic to many kinds of insects at doses applicable with micro-capillary tubes. In particular, we have found houseflies, *Musca domestica*, of the P.I.L. standard laboratory strain to be tolerant of *cyclo*hexanone applied at 0·2 μl./fly. On the other hand, Kocher & Ascher (1954) found that the same dosage gave a complete mortality of flies of an insecticide-resistant strain.

Care was needed to ensure that doses were fully retained by anaesthetized insects. For example, a prone beetle under anaesthesia was liable to lose to the surface on which it lay some of a dose put on its mesoscutellum, owing to liquid flowing round

the fold between the pro- and mesothoraces (compare satisfactory mesoscutellary treatment by the technique of Hewlett, 1954). However, beetles of several species when supine were dosed satisfactorily between the hind coxae (e.g. *Tribolium* spp., *Alphitobius laevigatus* (F.)) or on the abdominal sternites (*Calandra* spp.). Setae on flies under anaesthesia hold their bodies out of close contact with the surface supporting them, so helping to prevent loss of topically applied doses.

We are indebted to Mr F. P. W. Winteringham and Dr K. Williams for radiometric measurements to determine the reproducibility of dose. Miss J. Rider gave technical assistance. Mr M. A. Cordaroy constructed the holders for the microcapillaries and the anaesthetization table.

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SOME FIELD HABITATS OF MITES OF STORED FOOD PRODUCTS

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(With 3 Text-figures)

Forty-seven samples were collected from stacks of hay, corn and straw and from permanent grassland in the East Midland counties of England. The living mites were extracted and all except the Oribatei were identified. Records were kept of the occurrence of those mites associated with stored food products, of the mites *Forcellinia fungivora* Oud. and *Alliphis halleri* Can., and of the beetle *Enicmus minutus* L.

Fifty-two species of mites were identified of which twenty-two were associated with stored food and food stores. Twenty-one stored-product species were collected from hay- and strawstacks and five from grassland. *Acarus siro* L., a major pest of stored cereals and cereal products, was the dominant mite in stacks and was also found in grassland samples. *Tyrophagus longior* Gerv. was similarly distributed and occurred almost as frequently. The motile hypopial stage of *Acarus siro* was found in 43 % of the stack samples; it was attached to mites, beetles and other invertebrates. Its distribution pattern resembled that of the fungus beetle *Enicmus minutus*. *Tyrophagus palmarum* Oud., an uncommon stored-product mite, was the dominant species collected from grassland samples.

It is concluded that: (*a*) all the mites commonly associated with foodstuffs stored indoors are present in the outdoor habitat afforded by stacks; (*b*) *Acarus siro* and *Tyrophagus longior* are dominant in stacks and both appear to be field dwellers; (*c*) *T. palmarum* appears to be a successful field dweller and its occasional association with stored food may be accidental; (*d*) the dominant warehouse partnership of *Acarus siro* and *Glycyphagus destructor* does not occur outdoors; here *Tyrophagus longior* replaces *Glycyphagus destructor*; (*e*) species of the family Glycyphagidae appear to live mainly in haystacks sited in stackyards; and (*f*) considering how the mites were acquired, grainstacks standing in the open field have a peculiar combination of dominants, namely: *Acarus siro*, *Tyrophagus longior*, *T. palmarum* and *Alliphis halleri*.

Introduction

In Britain, food, especially cereals and cereal products, is liable to become infested by mites after short periods of storage. The species of mites concerned can be classified into four groups, according to their food (Hughes, 1948), as follows: (*a*) those that live on the stored material, almost all of which will belong to the family Tyroglyphidae Zachvatkin; (*b*) those that prey on the tyroglyphid mites and, in the main, belong to the families Cheyletidae Leach, Bdellidae Dugés, and Tydeidae Kramer, and to the superfamily Parasitoidea Evans; (*c*) a small number of mites that feed on minute fungi; (*d*) mites that are parasitic on the insects and rodents which may be present in food stores and stored food. Solomon (1943, 1944) has

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surveyed the published information in this field. The occurrence and distribution of stored-product mites in outdoor habitats has received very little attention except in Russia, where Sorokin (1951) has recorded eight species of granary mites living in the soil. Rodionov (1937) surveyed fields and meadows in a number of provinces in the Soviet Union and revealed the presence in them of all the common grain mites. Sorokin (1948) reported that haystacks of two provinces of western Russia harboured *Acarus siro* and *Tyrophagus longior* and that these were the most abundant of several species of cereal mites.

From time to time, a mite, or mites, associated with stored food products, is listed in a British paper dealing with a topic of soil zoology. For example, Sheals (1956) found *Acarus siro*, *Tyrophagus putrescentiae* (= *T. castellanii* Hirst) and *Glycyphagus destructor* to be common in soil samples taken from old grassland at Bellahouston Park, Glasgow.

Whilst investigating mite infestations of newly harvested grain in the U.K., entomologists have found conditions which indicated that the infestation was probably introduced from the field. They have also found very heavy mite infestations (principally *Tyrophagus* spp.) in haystacks in different parts of the country. Infestations of this type, and a few cases where the mite *Acarus siro* L. has occurred in fields, are reported by Robertson (1955).

Methods and materials

Sampling was done in the counties of Nottinghamshire, Derbyshire, Leicestershire, Lincolnshire (Kesteven, Lindsey, and one sample from Holland), Rutland, Northamptonshire and Buckinghamshire.

Two distinct habitats were sampled: stacks, and grassland which had not been ploughed for many years. These two habitats can be subdivided into:

	Type	Location
Stacks	Grain (sheaved) Straw (baled or loose) Hay (baled or loose)	Standing in the open field, or in a permanent stack-yard
Grassland	Roadside verges Permanent parkland pasture	Open country, as far away as possible from stored or growing cereal crops

Samples were collected in tins 6 in. high and 4 in. in diameter from March 1956 to July 1957, during all seasons of the year. Each tin was sealed and labelled as soon as the sample had been collected and before re-use was thoroughly cleaned with hot water, heat dried and inspected for freedom from live mites.

Stacks are generally built on a platform of dunnage (e.g. wooden spars, small tree trunks, brushwood). When the sheaf, bale or loose material is laid on this dunnage, it overlaps the platform and hangs down to touch, or almost touch, the ground. Whenever possible, stack samples were collected from beneath this overhang. When an overhang did not exist, as with some baled strawstacks, the sample was collected from beneath loose material lying at the base of the stack.

Each stack was sampled by the same method. Using the lid of the sampling tin, a small quantity of material was scraped from ground level at eight points on the periphery of the stack so that, as far as possible, each side of the stack was sampled twice. The quantities collected from these eight points were sufficient to fill one

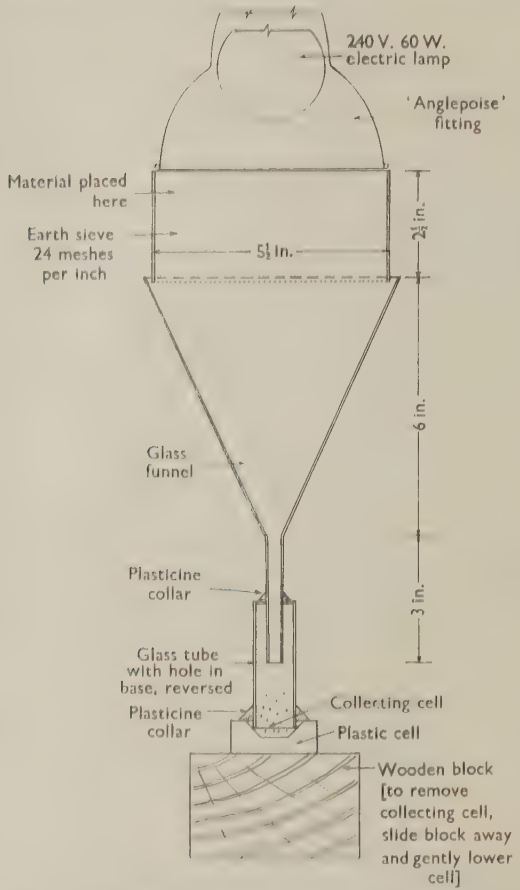

Fig. 1. The apparatus used for collecting mites from samples.

container. Material was always collected from ground level and never from the stack itself. The reliability of the method of sampling was checked in a pilot experiment for the study of seasonal fluctuations in population. Eight species were isolated from the first sample which was collected in October 1956 and the same eight species were taken from subsequent samples collected in January, February, May and July 1957.

Samples from roadside verges and permanent pastures were collected less

systematically. When the sampling area had been selected, the container was filled from at least ten places over a wide area with any loose material which appeared to be a likely harbourage for mites. Such material usually consisted of top-soil, dead

TABLE 1. *A complete list of identified Acarina: data of the occurrence and distribution of the mites associated with stored food products, and certain other species*

A. The twenty-two stored product species, and two other common species.

Species	% occurrence in grassland samples	% occurrence in stack samples	Distribution in stacks: Stacks sited in open fields	Stacks sited in yards	Grainstacks	Haystacks	Strawstacks
Suborder SARCOPTIFORMES							
Superfamily ACARIDIAE							
Tyroglyphidae							
1. *Acarus siro* L. 1758	23	83	×	×	×	×	×
2. *Tyrophagus longior* Gervais, 1844	17	76	×	×	×	×	×
3. *T. putrescentiae* (Schrank, 1781)	0	10	×	—	×	—	×
4. *T. palmarum* Oudemans, 1924	70	33	×	—	×	×	×
5. *Tyrolichus casei* (Oudemans, 1910)	0	*	—	×	—	—	—
6. *Rhizoglyphus echinopus* (Fumouze & Robin, 1868)	11	0	—	—	—	—	—
7. *Acotyledon munroi* Hughes, 1948	0	*	—	—	—	—	
8. *Forcellinia fungivora* (Oudemans, 1932)	29	13	×	×	×	×	×
Glycyphagidae							
9. *Glycyphagus destructor* (Schrank, 1781)	0	10	×	×	×	×	×
10. *G. domesticus* (de Geer, 1778)	0	*	—	×	—	×	—
11. *Blomia freemani* Hughes, 1948	0	6	×	×	—	×	—
12. *Ctenoglyphus plumiger* (Koch, 1835)	0	13	—	×	—	×	—
13. *C. canestrinii* (Armanelli, 1887)	0	6	—	×	—	×	—
Suborder PROSTIGMATA							
Cheyletidae							
14. *Cheyletus eruditus* (Schrank, 1781)	0	23	×	×	—	×	×
15. *C. trouessarti* Oudemans, 1902	0	*	—	×	—	×	—
16. *Cheletomorpha lepidopterorum* (Shaw, 1794)	0	10	×	×	—	×	×
Suborder MESOSTIGMATA							
Superfamily PARASITOIDEA							
Laelaptidae							
17. *Eulaelaps stabularis* (Koch, 1840)	0	40	×	×	×	×	×
18. *Hypoaspis casalis* Berlese, 1886	11	16	×	×	×	×	×
19. *H. smithii* Hughes, 1948	0	*	×	—	—	—	×
20. *Proctolaelaps hypudaei* Oudemans, 1902	0	16	×	×	×	×	—
Eviphididae							
21. *Alliphis halleri* (G. & R. Canestrini, 1881)	23	43	×	×	×	×	×
Phytoseiidae							
22. *Kleemania plumosa* (Oudemans, 1902)	0	26	×	×	—	×	×
23. *K. plumigera* (Oudemans, 1930)	0	6	×	×	—	×	×
24. *Melichares agilis* Hering, 1838	0	6	×	—	×	×	—

* Occurred in one sample only.

TABLE 1 (*cont.*)

B. Other species.

Suborder SARCOPTIFORMES	
Superfamily ACARIDIAE	
Tyroglyphidae	25. *Calvolia* sp.
Glycyphagidae	26. *Xenoryctes krameri* (Michael, 1886)
	27. *Glycyphagus* sp.nov.
Anoetidae	28. *Anoetus sapromyzarum* (Dufour, 1839)
Suborder PROSTIGMATA	
Tydeidae	29. *Tydeus* sp.
Tetranychidae	30. *Bryobia praetiosa* Koch, 1836
Raphignathidae	31. *Raphignathus* sp.
Scutacaridae	32. *Scutacarus* sp.
Suborder MESOSTIGMATA	
Superfamily PARASITOIDEA	
Laelaptidae	33. *Hypoaspis* sp.
	34. *Cosmolaelaps claviger* (Berlese, 1883)
	35. *Pseudolaelaps doderoi* Berlese, 1910*
Eviphididae	36. *Eviphis ostrinus* (Koch, 1836)*
Phytoseidae	37. *Typhlodromus rhenanus* (Oudemans, 1905)
	38. *Seiulus* sp.
	39. *Ameroseius corbicula* (Sowerby, 1806)*
Parasitidae	40. *Pergamasus* sp.
	41. *Eugamasus loricatus* (Wankel, 1861)
	42. *Parasitus* sp.
	43. *Holoparasitus* sp.
Machrochelidae	44. *Machrocheles matrius* (Hull, 1925)
	45. *M. glaber* (Muller, 1860)
Veigaiaidae	46. *Veigaia serrata* Willmann, 1935
Neoparasitidae	47. *Epicriopsis horridus* (Kramer, 1876)
Pachylaelaptidae	48. *Pachylaelaps* sp.
Poecilochiridae	49. *Poecilochirus necrophori* Vitzthum, 1930
Rhodacaridae	50. *Rhodacarus roseus* Oudemans, 1910*
Superfamily UROPODOIDAE	
Urodinychidae	51. *Urodinychus* sp.
	52. *Leiodinychus* sp.

* Species identified by D. Macfarlane and E. Browning of the British Museum (Natural History).

leaves, and green vegetation in a fairly advanced stage of decay. The reliability of this method of sampling was not tested.

In the laboratory, each sample was placed in a home-made, modified Berlese-type funnel (Fig. 1) and left for a period of 6 hr., a time that appeared to be long enough to ensure that practically all live insects, mites and other creatures were driven from the sample. Most samples were placed in the funnel on the day following collection, but some were left in their containers for 2 days. The funnel was dismantled and thoroughly cleaned at the end of each exposure period.

The apparatus was designed so that mites were collected alive, with little risk of escape. The collecting cell, after removal from the apparatus, was placed in the centre of a Petri dish previously ringed with a wide band of lactic acid to prevent the escape of insects and of the more active mites which scaled the sides of the cell. Specimens were mounted directly in 60% lactic acid on numbered slides.

Determinations were made according to Hughes (1948) and Baker & Wharton (1952). All mites belonging to those families known to be associated with stored food products were identified to species; others were determined to genera or species but the Oribatei were not identified. Any specimen which could not be identified with certainty was sent to Dr A. Margaret Hughes. A complete list of the identified mites, based on the classification of Baker & Wharton (1952) and Evans (1957), is given in Table 1.

Results

From seventeen grassland samples and thirty stack samples, fifty-two species of mites, distributed in forty-one genera and twenty-three families, were identified (Table 1). According to the information given by Hughes (1948) and the records of the Infestation Control Division, Ministry of Agriculture, Fisheries and Food, twenty-two of these species are associated with stored food products and food stores (Table 1, Section A).

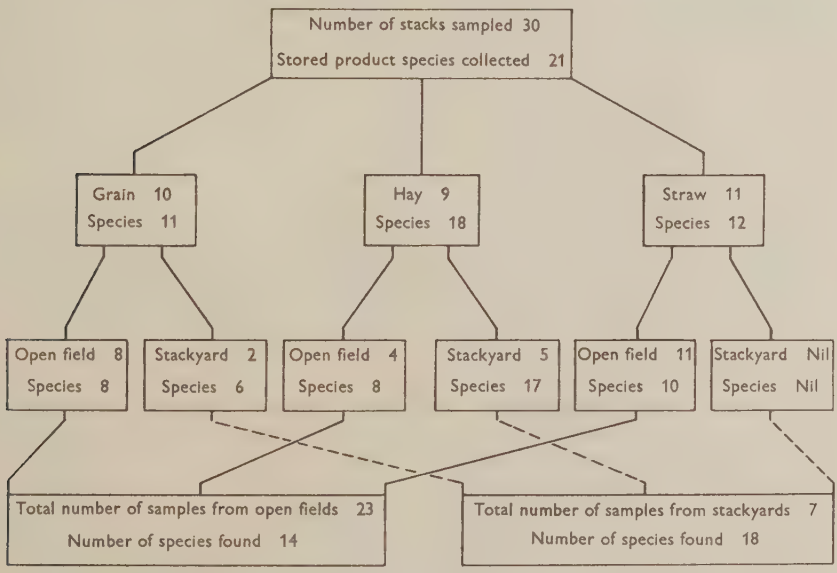

Fig. 2. The distribution of species of mites according to habitat.

The seventeen collections from grassland yielded five stored-product mites; they were: *Acarus siro*, *Tyrophagus longior* (= *T. tenuiclavus*), *T. palmarum* (= *T. perniciosus*), *Hypoaspis casalis* and *Rhizoglyphus echinopus*.

The thirty collections from thirty stacks gave a total of twenty-one different species of stored-product mites (Fig. 2). *R. echinopus* was not found in the stack samples.

The species and groups of stored-food mites commonly found were:

(1) *Acarus siro* (Fig. 3*a*, *b*).

This species ranked first in frequency of occurrence and was found in 83% of all the stacks sampled (Fig. 3*a*). In the collections from grassland it ranked second, being present in 23% of the seventeen samples.

(2) The motile hypopial stage of *A. siro*.

This important stage in the life-cycle of *A. siro* was found regularly in all types of samples (Fig. 3*c*). The shape of the histogram is similar to that for the fungus beetle *Enicmus minutus* (Fig. 3*i*). Whenever hypopi and *E. minutus* adults occurred together, the beetles were always carrying a considerable number of hypopi. The hypopi were also recorded on other beetles, beetle larvae, moth larvae, mites and spiders. Sometimes the carrier had one or two passengers and, at other times, it was smothered in hypopi to such a degree that it could not move. A sample, taken from a haystack standing in an open field, contained so many hypopi that a rough count was made. It was estimated that there were 10,000 hypopi in 30 g. of material. The hypopal stage also occurred in 23% of the seventeen grassland collections. On two occasions it was the only stage of *Acarus siro* present in the sample.

(3) *Tyrophagus longior* (= *T. tenuiclavus*) (Fig. 3*d*).

This mite was second in frequency of occurrence in stacks. Its histogram is remarkably similar to that of *Acarus siro*.

(4) *Tyrophagus palmarum* (= *T. perniciosus*) (Fig. 3*e*).

T. palmarum ranked first in frequency of occurrence in grassland but was less common in stacks. It occurred in 43% of samples collected from stacks situated in the open field but was not found in stackyard samples. The shape of the histogram indicates that this mite is primarily a soil or grassland dweller. It is uncommon in stored threshed cereals and cereal products.

(5) Family Glycyphagidae (Fig. 3*f*).

Members of this family were not found in grassland samples. Six species were collected from stacks, including an undescribed species of the genus *Glycyphagus*. Five of the six species were confined to haystacks and, with one exception, these haystacks were standing in permanent stackyards. *G. destructor* was the exceptional member of the family, being found in one sample from each type of stack. The Glycyphagidae occurred in 23% of all stacks, the highest individual occurrence being of *Ctenoglyphus plumiger* which was found in four out of thirty samples.

(6) Family Cheyletidae.

Three species of this family were recorded in stacks but not from grassland samples. *Cheyletus trouessarti* was found once. *Cheletomorpha lepidopterorum*, slightly more common in food stores, was found three times. The mite *Cheyletus eruditus* was collected from seven out of thirty stacks. This mite is commonly encountered in food-storage premises, often occurring in such large numbers as to control the Tyroglyphidae (Norris, 1958).

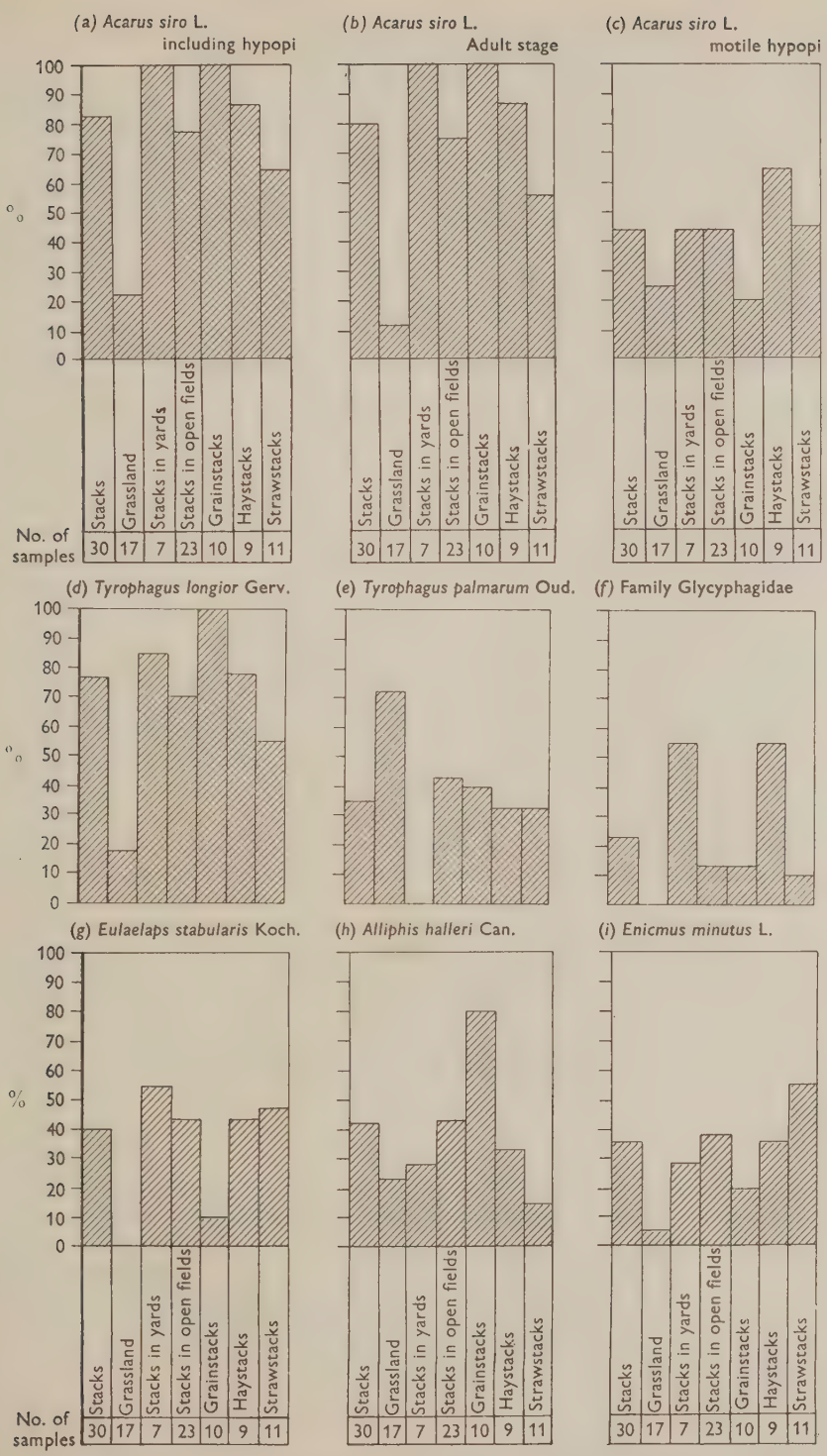

Fig. 3. The frequency of occurrence of certain species of mites in different habitats.

(7) Suborder Mesostigmata

Seven mites from this suborder were placed in the stored-product group. Only one, *Hypoaspis casalis*, was recorded from grassland samples; the others were confined to stacks. *Eulaelaps stabularis* (Fig. 3*g*) ranked third in frequency of occurrence for all the stored-product mites, and ranked first of the combined parasitic and predacious groups. It occurred in all types of stacks, but was not recorded from grainstacks in the open field. *E. stabularis* is the most common of the mesostigmatid mites found associated with granary-stored grain.

The species of stored-product mites occurring occasionally included the two species, *Tyrophagus putrescentiae* (= *T. castellanii*) and *Glycyphagus destructor*, which were found in 10% of the stacks only. Nine of the twenty-one species collected from stacks were recorded in less than 10% of the samples. They were: *Acotyledon munroi*, *Cheyletus trouessarti*, *Tyrolichus casei*, *Hypoaspis smithii*, *Glycyphagus domesticus*, *Kleemania plumigera*, *Ctenoglyphus canestrinii*, *Melichares agilis*, *Blomia freemani*. These nine species are not common in food-storage premises, but some are common on particular types of food (e.g. *Tyrolichus casei* on high-protein material, *Melichares agilis* on dried fruit).

The presence of two species, not considered to belong to the stored-product group, was recorded in the forty-seven samples. The occurrence of *Forcellinia fungivora* was noted because it was impossible to distinguish between this mite and *Tyrophagus* in live material. Only under high magnification can mounted specimens be distinguished from *T. longior*. *Forcellinia fungivora* ranked second in frequency of occurrence for grassland samples (29%) and was taken in stacks in the open field three times, and in stackyards once. Because the deutonymph is associated with rodent nests, *Alliphis halleri* was collected from samples in the hope that its distribution would help to show what part rodents play as carriers of stored-product species. It was found in all types of stacks and situations (Fig. 3*h*). It occurred in 80% of the samples taken from ten separate grainstacks, the majority of which were sited in open fields.

The fungus beetle *Enicmus minutus* (Fig. 3*i*) was found occasionally. This insect is common in food-storage premises of all kinds and has been observed to act as a carrier for the motile hypopus of *Acarus siro* whenever they are recorded together. During this survey, they were recorded together in stack samples on eight occasions; *Enicmus minutus* acted as a carrier each time. The beetle was present in 36% of stack samples and occurred most frequently in samples taken from strawstacks (55%).

Discussion

Of the twenty-four species for which records were kept, only two occurred more frequently in grassland samples than in stack samples, namely, *Tyrophagus palmarum* and *Rhizoglyphus echinopus*. The latter is common in soil.

The mite *Tyrophagus palmarum* was the dominant species in grassland samples and ranked fourth in stack samples. *T. palmarum* is not one of the common stored-products mites, but has been recorded in silo-stored grain three times, on cheese

once, and the author has found it in debris taken from a number of beehives. Since it was not found in stackyards, but did occur in 43% of stacks in the open field, it would appear to be a field dweller which becomes associated with stored foods by accident.

Among mites of stored foods are two species which are common and universally distributed in storage premises, *Acarus siro* and *Glycyphagus destructor*. In warehouses these two mites are frequently found in association with one another, but this partnership does not appear to extend to outdoor habitats. In stacks, the dominant partners seem to be *Acarus siro* and *Tyrophagus longior*. The reason why *Acarus siro* has different partners in different habitats, or alternatively, why *A. siro* can succeed in both habitats and its partners fail, is not known. The two species *A. siro* and *Tyrophagus longior*, dominant partners in the stacks of eastern England, are also the dominant partners in the haystacks of parts of west Russia (Sorokin, 1948).

There is some evidence that the distribution of certain stored-product species is limited by the siting or composition of stacks, or by both these factors. Members of the family Glycyphagidae seem to live mainly in haystacks which are situated in stackyards, but this is a tentative conclusion which may have to be modified when further work has been done.

Although only eight grainstacks in open fields were sampled it is noteworthy that four mites occurred very commonly; these were *Acarus siro* (100%), *Tyrophagus longior* (87%), *T. palmarum* (50%) and *Alliphis halleri* (80%).

A. halleri may have been introduced soon after the stacks were built, when the autumn migration of rodents occurred. *Tyrophagus palmarum* is, according to this survey, primarily a mite which lives in fields. This species may have been introduced in the sheaf when the stack was made, particularly if the cereal was used as a nurse crop for a temporary ley. *Acarus siro* and *Tyrophagus longior* could have been introduced in one or both of these ways. They may have travelled on the body of a rodent, on a sheaf, or on both.

The most important fact which has emerged from this preliminary survey is that the mite *Acarus siro* was found to be common in all types of stacks, wherever they were sited. It may therefore commonly be present in places far removed from food stores. *A. siro* is the only mite classified as a major pest of stored cereals and cereal products and a study of its distribution and methods of travelling to and from indoor and outdoor habitats would contribute towards the improvement of control measures at present employed in storage premises.

I wish to thank Dr J. A. Freeman, Dr A. M. Hughes and Dr I. Thomas for much helpful criticism of the manuscript. I am greatly indebted to Dr A. M. Hughes for many determinations. I am also indebted to D. Macfarlane and E. Browning for the identification of some Mesostigmatid mites, and to A. G. Jenson for the illustrations.

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FIELD STUDIES ON WHEAT-BULB FLY INFESTATIONS

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An account is given of two experiments done on commercial farms in Essex to study the effect of tilth, cultivation and seed rate on wheat-bulb fly infestation and the effect of infestation on yield. Heavier infestations developed on rough fallows and those cultivated during the oviposition period, than on smooth fallows and those not cultivated during the oviposition period. These results confirmed those of an earlier experiment done at Rothamsted. At each level of infestation, more larvae survived on plots sown at 3 bus./acre than on plots sown at $\frac{2}{3}$ or 1 bus./acre. Although some plots had a high larval population no effect of infestation on yield was observed even at the lower seed rates. This is attributed to early sowing. The extent to which the cultural treatments studied can be used to control wheat-bulb fly infestations is discussed.

Introduction

Soil conditions during the oviposition period greatly affect wheat-bulb fly infestations (Raw, 1955). In a factorial experiment on fallow land at Rothamsted, plots with a rough tilth contained more eggs than those with a smooth tilth and plots cultivated during the oviposition period contained more than undisturbed plots. The different levels of infestation established in this way were used to study the effect of infestation on yield (Raw & Lofty, 1957). Plant counts on Broadbalk wheat plots (Raw, 1954) suggested that the survival of wheat-bulb fly larvae after hatching may be affected by the numbers of wheat plants and shoots available for infestation, but the evidence was inconclusive as it was based on observations made on plots which differed in other ways than in plant and shoot density.

To obtain further information about these aspects of wheat-bulb fly infestation, two field experiments were done on commercial farms in Essex where heavy infestations often occur.

Sites and experimental layout

The experiments were at Fowes Farm, Gt. Yeldham and at Herkstead Hall, Cornish Hall End, in fields bare-fallowed in summer 1957 and adjacent to infested wheat crops. The soil type at each farm is boulder clay overlying chalk.

At each site, four treatments were applied to the fallow during the oviposition period. They were: (1) undisturbed rough tilth, screened with mosquito netting to prevent oviposition; (2) undisturbed smooth tilth; (3) undisturbed rough tilth; (4) rough tilth cultivated at 7- to 10-day intervals.

Each experiment was drilled with wheat at two seed rates to give contrasted plant and shoot densities in spring. At Fowes Farm the seed rates were 3 and $\frac{2}{3}$ bus./acre, at Herkstead Hall they were 3 and 1 bus./acre.

These experiments were laid out as five randomized blocks of four whole plots.

Fallow treatments were applied to whole plots and seed rates on subplots. Whole plots were 10 ft. long and two drills (each twenty coulters) wide at Fowes Farm and four drills (each fifteen coulters) wide at Herkstead Hall.

Application of treatments. Each site was taken over in early July after it had been tractor-cultivated by ploughing and disking to give a rough tilth. A smooth tilth was prepared on the appropriate plots using harrows and a Cambridge roller. The treatment resulted in some consolidation but was only partially effective in breaking down the tilth because of continued dry weather. Nevertheless, it produced a tilth that contrasted with the rough plots.

The screens, made from fine Terylene mosquito netting, were 21×6 ft. with canvas sides 1 ft. high. Each was supported on a wire framework and placed centrally on the appropriate plots. At Herkstead Hall they were erected during the first week in July and at Fowes Farm during the second week. They remained in place throughout the oviposition period. Unfortunately they were torn by birds and probably by hares and rabbits. Various ways for protecting them were tried without success and, although they were repaired frequently, oviposition was not completely prevented.

The plots with rough cultivated tilth were hand-forked to a depth of 4–6 in. on five occasions at 7- to 10-day intervals between 19 July and 20 August. To avoid breaking down the rough tilth the cultivations were done during periods of dry weather, when fresh soil could be exposed and the rough tilth maintained.

At Herkstead Hall, weeds were particularly troublesome on the smooth plots and to a less extent on the plots with rough undisturbed tilth. The smooth plots were hand-weeded in mid-August and later, when the screens were removed, all the plots were hand-weeded. Few weeds grew at Fowes Farm but the plots were hand-weeded when the screens were removed.

The experiment at Fowes Farm was drilled on 3 October 1957 with Cappelle wheat treated with a mercury seed dressing. A compound granular fertilizer 5% N; 12% P_2O_5; 12% K_2O was combine-drilled with the seed at 3 cwt./acre. At Herkstead Hall the plots were drilled on 10 October 1957 using similar seed and compound fertilizer at $2\frac{1}{2}$ cwt./acre.

Experimental methods

Eggs. The effect of the fallow treatments on oviposition was measured by estimating the egg population in the soil at the end of the oviposition period. Between late August and mid-October, twenty soil samples each $2\frac{1}{2}$ in. diameter and 8 in. deep were taken from each plot at both sites. The wheat-bulb fly eggs were extracted from the samples by a standard flotation method and the fertile ones separated from the sterile.

To estimate the egg population just before hatching, twelve soil samples were taken on 7 February from all except the screened plots. The eggs recovered from these samples were given to Mr M. J. Way for laboratory experiments and he separated the non-viable eggs (sterile or dead) from the remainder. In this way mortality from these causes was estimated.

TABLE 1. *Wheat-bulb fly population estimates*

(Thousands per acre.)

Fallow treatment	Egg counts: Sept.–Oct. 1957 All eggs	Egg counts: Sept.–Oct. 1957 Viable eggs	Egg counts: Feb. 1958 All eggs	Egg counts: Feb. 1958 Viable eggs	Plant counts (April 1958): Plants 3 bus.	Plants ⅔ bus.	Shoots 3 bus.	Shoots ⅔ bus.	Larvae 3 bus.	Larvae ⅔ bus.	Pupal counts (May–June 1958) Pupae 3 bus.	Pupae ⅔ bus.
Fowes Farm												
Rough tilth screened	639	465	—	—	874	321	4085	2001	96	24	0	2
Smooth tilth	784	543	645	459	881	284	4227	2001	293	84	69	40
Rough tilth	1859	1395	1944	1776	993	231	4794	1232	568	63	164	43
Rough tilth cultivated	3391	2968	2613	2190	963	418	4653	2554	860	382	390	77
Herkstead Hall					3 bus.	1 bus.	3 bus.	1 bus.	3 bus.	1 bus.	3 bus.	1 bus.
Rough tilth screened	183	153	—	—	1045	321	3689	1404	42	27	31	9
Smooth tilth	214	118	175	113	739	366	2688	1486	42	19	11	18
Rough tilth	701	604	738	680	866	329	3196	1464	229	126	171	66
Rough tilth cultivated	1426	1374	1492	1123	971	418	3316	1561	378	216	215	163

Larvae. The effect of the seed rates on larval survival was measured by estimating the larval population in the plants at the beginning of April, i.e. when plant infestation was thought to be complete but subsequent mortality small. At each site, two samples, each consisting of the plants from a 6 in. length of two adjacent rows, were taken from each subplot on 1 April 1958. The plants were measured, all the shoots were dissected and the numbers of plants, shoots, infested shoots and larvae were recorded for each sample. As the infestation at Herkstead Hall was lower than at Fowes Farm, a second set of plant samples was taken there on 17 April 1958 to estimate the population better.

Pupae. To supplement the estimates of larval population and to provide an estimate of the effect of seed rate on the emergent fly population, the plots were sampled for pupae in late May and early June. At Fowes Farm four soil samples, each 4 in. diameter and 3 in. deep, were taken on the rows from each subplot. At Herkstead Hall eight such samples were taken from each subplot. Pupae were extracted from the samples by the method used to extract eggs.

Crop yield. An area 1 yd. wide and seventeen rows (10 ft.) long, corresponding in position to the centre of the areas covered by the screens, was harvested by hand from each subplot and threshed on a small-scale threshing machine.

RESULTS

Population estimates derived from the soil and plant samples are summarized in Table 1. The estimates of eggs, larval and pupal populations are based on a square-root transformation of the sample counts; estimates of plant and shoot densities are based on untransformed data.

TABLE 2. *Effect of fallow treatments on egg population*

(Plot means.)

	Fowes Farm		Herkstead Hall	
Fallow treatment	Autumn count* √(all eggs)	Spring count† √(all eggs)	Autumn count* √(all eggs)	Spring count† √(all eggs)
Rough tilth screened	3·16	—	1·69	—
Smooth tilth	3·50	2·46	1·83	1·28
Rough tilth	5·39	4·27	3·31	2·63
Rough tilth cultivated	7·28	4·59	4·72	3·74
S.E. of means	±0·24	±0·22	±0·45	±0·30

* Twenty samples per plot. † Twelve samples per plot.

Effect of fallow treatments on egg population. The data from the egg counts (all eggs) were analysed using a square-root transformation. Table 2 gives the plot means and standard errors. At each site, plots with a rough cultivated tilth contained significantly more eggs than plots with a rough undisturbed tilth which contained significantly more than plots with a smooth undisturbed tilth. The spring counts agreed with the autumn counts.

TABLE 3. *Effect of fallow treatments and seed rate on larval populations*

(Plot means (numbers per foot of drill).)

Fowes Farm

Fallow treatment	Plants/ft. 3 bus.	Plants/ft. ⅔ bus.	Plants/ft. Means	Plants/ft. Diffs.	Shoots/ft. 3 bus.	Shoots/ft. ⅔ bus.	Shoots/ft. Means	Shoots/ft. Diffs.	√(Larvae/2 ft.) 3 bus.	√(Larvae/2 ft.) ⅔ bus.	√(Larvae/2 ft.) Means	√(Larvae/2 ft.) Diffs.
		±1·00*	±0·78	±1·25		±4·61*	±3·60	±5·74		±0·45*	±0·31	±0·65
Rough tilth screened	11·7	4·3	8·0	+ 7·4	54·7	26·8	40·8	+27·9	1·6	0·8	1·2	+0·8
Smooth tilth	11·8	3·8	7·8	+ 8·0	56·6	26·8	41·7	+29·8	2·8	1·5	2·1	+1·3
Rough tilth	13·3	3·1	8·2	+10·2	64·2	16·5	40·4	+47·7	3·9	1·3	2·6	+2·6
Rough tilth cultivated	12·9	5·6	9·3	+ 7·3	62·3	34·2	48·3	+28·1	4·8	3·2	4·0	+1·6
Means	12·4	4·2	8·3	+ 8·2±0·61	59·4	26·1	42·7	+33·4±2·86	3·3	1·7	2·4	+1·6±0·31

Herkstead Hall

Fallow treatment	Plants/ft. 3 bus.	Plants/ft. 1 bus.	Plants/ft. Means	Plants/ft. Diffs.	Shoots/ft. 3 bus.	Shoots/ft. 1 bus.	Shoots/ft. Means	Shoots/ft. Diffs.	√(Larvae/4 ft.) 3 bus.	√(Larvae/4 ft.) 1 bus.	√(Larvae/4 ft.) Means	√(Larvae/4 ft.) Diffs.
		±0·78*	±0·56	± 1·09		±3·06*	±2·48	± 3·55		±0·30*	±0·26	±0·25
Rough tilth screened	14·0	4·3	9·1	+ 9·7	49·4	18·8	34·1	+30·6	1·5	1·2	1·4	+0·3
Smooth tilth	9·9	4·9	7·4	+ 5·0	36·0	19·9	28·0	+16·1	1·5	1·0	1·3	+0·5
Rough tilth	11·6	4·4	8·0	+ 7·2	42·8	19·6	31·2	+23·2	3·5	2·6	3·0	+0·9
Rough tilth cultivated	13·0	5·6	9·3	+ 7·4	44·4	20·9	32·6	+23·5	4·5	3·4	4·0	+1·1
Means	12·1	4·8	8·4	+ 7·3±0·54	43·2	19·8	31·4	+23·4±1·77	2·7	2·1	2·4	+0·7±0·11

* S.E. for use in vertical comparisons only.

The mosquito netting screens failed to prevent oviposition, probably because they were torn frequently during the oviposition period, though some eggs may have been carried into the screened plots by cultivations when preparing the seedbed. At each site the egg population of the screened plots was lower, though not significantly so, than that of the smooth plots, but the larval and pupal counts (see below) suggest that it was overestimated.

Effect of fallow treatments and seed rate on larval population. Table 3 shows the plot means and standard errors for the numbers of plants, shoots and larvae per foot of drill. The larval population for each fallow treatment reflects the effect of the fallow treatments on the egg population. The whole plot means show that, at each site, plots with rough cultivated tilth contained significantly more larvae than other plots. At Fowes Farm the larval populations of the plots with rough and with smooth undisturbed tilth did not differ significantly, largely because of the low population estimate for the subplots with rough tilth sown at $\frac{2}{3}$ bus./acre where plant and shoot density was unusually low. At Herkstead Hall the larval population of plots with rough undisturbed tilth was significantly greater than that of the smooth undisturbed plots. At Fowes Farm the screened plots had significantly fewer larvae than the other plots, but at Herkstead Hall their population was equal to that of the plots with smooth undisturbed tilth. At Fowes Farm the mean plant and shoot density per foot on subplots sown at 3 bus./acre was 12·4 and 59·4 compared with 4·2 and 26·1 on subplots sown at $\frac{2}{3}$ bus./acre, i.e. a ratio of 3:1 for plants and 2·3:1 for shoots. At Herkstead Hall the corresponding plant and shoot densities were 12·1 and 43·2 on subplots sown at 3 bus./acre and 4·8 and 19·8 on subplots sown at 1 bus./acre, i.e. a ratio of 2·5:1 for plants and 2·2:1 for shoots. The plant ratios were lower than expected from the seed rates, probably due to inaccuracy in calibrating the drills for the lower seed rate. The shoot ratios were lower than the plant ratios and indicate greater tillering at the lower seed rate.

At each site the mean larval population was significantly greater in the subplots sown at 3 bus./acre than in those sown at the lower seed rate. This effect was consistent at each level of infestation, as the interaction between seed rate and fallow treatment was not significant at either site ($F = 1{\cdot}47$, $n_1 = 3$, $n_2 = 16$ at Fowes Farm; $F = 2{\cdot}12$, $n_1 = 3$, $n_2 = 16$ at Herkstead Hall). Clearly, more larvae survived in subplots sown at the higher seed rate.

Effect of fallow treatments and seed rate on pupal population. The results in Table 4 support those in Tables 2 and 3. The significant differences in pupal population corresponding to the fallow treatments reflect the effect of these treatments on the egg population, and the significant differences in the pupal population corresponding to the seed rates reflect the effect of the seed rates on larval survival.

Effect of infestation on yield. Table 5 summarizes the grain yields.

At Fowes Farm no effect of infestation on yield was observed ($F = 0{\cdot}31$, $n_1 = 3$, $n_2 = 12$). Seed rate affected yield; the mean difference of $3{\cdot}3 \pm 1{\cdot}54$ cwt./acre was just significant ($P < 0{\cdot}05$) and was consistent at each level of infestation, as the interaction between seed rate and infestation was not significant ($F = 2{\cdot}68$, $n_1 = 3, n_2 = 16$).

At Herkstead Hall the yields were affected by nitrogen deficiency on some plots, probably caused by weed growth on the fallows, and aggravated by an accidental delay in applying the spring dressing of nitrogen. In mid-April crop growth on all plots appeared normal. By mid-May growth on plots following rough cultivated fallow was satisfactory, but elsewhere there were symptoms of nitrogen deficiency, particularly on the subplots sown at 3 bus./acre. It was then discovered that the spring dressing of nitrogen had not been applied and, although a heavy dressing of

TABLE 4. *Effect of fallow treatments and seed rate on pupal population*

(Plot means)

	Fowes Farm √(Pupae/16 in. drill)			Herkstead Hall √(Pupae/32 in. drill)		
Seed rate ...	3 bus.	⅔ bus.	Mean	3 bus.	1 bus.	Mean
Fallow treatment	±0·33	±0·33	±0·23	±0·34	±0·34	±0·26
Rough tilth screened	0·00	0·20	0·10	1·05	0·55	0·80
Smooth tilth	1·11	0·85	0·98	0·63	0·80	0·71
Rough tilth	1·71	0·88	1·30	2·47	1·54	2·00
Rough tilth cultivated	2·64	1·17	1·91	2·77	2·41	2·59
Mean ±0·16	1·36	0·78	1·07	Mean ±0·15 1·73	1·32	1·52

TABLE 5. *Effect of infestation on yield*

(Plot yields (cwt./acre of grain at 85 % dry matter))

	Fowes Farm					Herkstead Hall				
	Larvae (thousands/ acre)		Yield			Larvae (thousands/ acre)		Yield		
Seed rate ...	3 bus.	⅔ bus.	3 bus.	⅔ bus.	Mean	3 bus.	1 bus.	3 bus.	1 bus.	Mean
Fallow treatment			±1·90	±1·90	±1·09			±1·43	±1·43	±1·23
Rough tilth screened	96	24	32·2	30·1	31·2	42	27	26·3	25·9	26·1
Smooth tilth	293	84	32·3	29·8	31·1	42	19	24·5	22·3	23·4
Rough tilth	568	63	32·7	27·1	29·9	229	126	28·7	27·6	28·1
Rough tilth cultivated	860	382	32·6	29·8	31·2	378	216	34·9	27·7	31·3
	Mean ±1·09		32·5	29·2	30·8	Mean ±0·51		28·6	25·9	27·2

nitro-chalk (6 cwt./acre) was immediately given to all plots, growth and yield were undoubtedly affected. The observed differences in crop yield are unrelated to the level of wheat-bulb fly infestation and appear to be related to the weediness of the preceding fallows, for yields were lowest after the smooth fallows which were weedy, intermediate after the undisturbed rough fallows which were less weedy and greatest after the cultivated rough fallows where weeds had been negligible. As the mean plot yields after undisturbed rough fallows were not significantly affected by screening (the difference due to screening was $-2{\cdot}0 \pm 1{\cdot}74$ cwt./acre)

the results indicate that infestation did not affect yield and so support those from Fowes Farm.

Seed rate affected yield, the mean difference of $2{\cdot}7 \pm 0{\cdot}72$ cwt. was significant ($P < 0{\cdot}01$), but this effect was not consistent at each level of infestation as the interaction between seed rate and infestation was also significant ($F = 4{\cdot}31$, $n_1 = 3$, $n_2 = 16$, $P < 0{\cdot}05$). The yields from subplots show that seed rate only affected yield significantly on the plots following rough cultivated fallow, i.e. where there was no nitrogen deficiency in spring. On these plots, the higher seed rate increased the yield by $7{\cdot}2 \pm 2{\cdot}02$ cwt./acre. Elsewhere the effect of seed rate was not significant, probably because of the greater effect of nitrogen deficiency at the higher seed rate.

Population survival rate. Survival rate at each stage can be estimated from Table 1. As the survival rate appears to be independent of the level of infestation, a mean survival rate for each stage was calculated by combining the data for the unscreened plots (Table 6).

TABLE 6. *Average population (thousands per acre) and survival rate of wheat-bulb fly*

Fowes Farm

	Eggs			Larvae		Pupae	
	Sept.–Oct. 1957		Feb. 1958	Seed rate			Overall survival
Total	2011	86%	1734	3 bus.	574	36% 208	10%
	81%	73%	85%	39%			
Viable	1635		1475	⅓ bus.	176	30% 53	2·7%
				12%			

Herkstead Hall

	Eggs			Larvae		Pupae	
	Sept.–Oct. 1957		Feb. 1958	Seed rate			Overall survival
Total	780	103%	802	3 bus.	216	61% 132	17%
	90%	82%	80%	34%			
Viable	699		639	1 bus.	120	68% 82	10·5%
				19%			

Between 80 and 90% of the eggs were viable and viability changed little during the winter. 86% or more of the eggs survived the winter, so 73–82% of all eggs present at the end of the oviposition period were viable just before hatching. Larvae from 34–39% of these eggs became established in plants on plots sown at 3 bus./

acre. On plots sown at $\frac{2}{3}$ or 1 bus./acre the corresponding survival rate was 12–19%. At Fowes Farm 30–36% of these larvae pupated and at Herkstead Hall 61–68% pupated. The overall survival from the end of oviposition to pupation ranged from 2·7 to 17%.

Discussion

The effect of tilth and cultivation on infestation confirmed the results of the experiment at Rothamsted (Raw, 1955). Although the average levels of infestation differed, tilth and cultivation affected infestation similarly in each experiment except that cultivation had more effect in the Essex experiments, where it was done more frequently, than at Rothamsted.

The experimental plots were small; those in Essex were 25 and 38 sq. yd. and those at Rothamsted were 3 sq. yd., so differences arising within very small areas were being measured. However, a comparable difference between large areas has been recorded. The experiment at Herkstead Hall was done in a 10-acre field, half of which was cultivated and harrowed in May and June in an unsuccessful attempt to prepare it for cropping. Both halves were cultivated later, but the effect of the early cultivation persisted and one-half of the field remained rougher than the other. Both halves were sampled separately for wheat-bulb fly eggs during July and August by F. E. Maskell of the National Agricultural Advisory Service, Trumpington, Cambridge. The infestation in the rougher half of the field was consistently higher than in the smoother and at the end of August, when oviposition had stopped, the infestations were 1,575,000 and 725,000 eggs per acre, respectively.

Several factors may account for the effect of tilth and cultivation on infestation. Wheat-bulb flies lay their eggs in cracks in the soil and both rough tilth and cultivation can be expected to provide more such oviposition sites than a smooth or uncultivated tilth. Another anthomyid, the bean-seed fly, *Chortophila cilicrura* (Rond.), is strongly attracted to freshly cultivated soil (Miles, 1950) and wheat-bulb fly may react similarly. Recent work on cabbage-root fly, *Chortophila brassicae* (Hughes, unpublished), shows that differences in infestation level, of the same order as those observed in the wheat-bulb fly experiments, can be caused by carabid beetles which prey on the eggs. The ecological conditions under which cabbage-root fly and wheat-bulb fly occur differ greatly and predators of wheat-bulb fly eggs have not been studied, but eggs laid in a rough tilth or buried by cultivation are likely to be better protected from predators than eggs laid in more exposed situations.

Seed rate affected the survival of larvae by affecting plant and shoot density. Stokes (1956) showed that wheat-bulb fly larvae are chemically attracted by exudates from wheat plants. This response no doubt assists newly hatched larvae to reach wheat plants. Plant density will affect survival by determining the strength and distribution of the chemical stimulus to which the larvae respond. Shoot density may contribute to this and will also affect the survival of larvae which have reached wheat plants. Two or more larvae are rarely found in one shoot (Gough, 1946) even in heavy infestations, and this suggests that larvae can detect and avoid infested shoots (Mellanby, 1958). The numbers of viable eggs in February 1958

give an estimate of the numbers of emerging larvae. The plant counts show that plant and shoot density affect larval survival even when there are more plants and shoots than emerging larvae, as on the subplots at Herkstead Hall following smooth tilth. They also show, by the consistent survival rate, that excess shoots do not compensate for shortage of plants when there are more larvae than plants. This suggests that, in these experiments, the effect of seed rate came mainly from plant density affecting larval survival. However, when there were more emerging larvae than either plants or shoots, as on the plots at Fowes Farm following rough tilth, then the survival rate was extremely low, 3·5%.

There is no satisfactory explanation for a greater proportion of larvae pupating at Herkstead Hall than at Fowes Farm. The pupal counts were made from few samples and so had large sampling errors. Larval mortality in the interval between the first and second set of plant samples taken at Herkstead Hall would reduce the estimate of the larval population and so increase the estimate of the proportion which pupated, but this seems insufficient to explain the large difference which was observed.

In spite of the heavy infestation on some plots, it produced no measurable effect on yield, no doubt because the plots were sown in early October and the plants were established and tillering well before the eggs hatched. The relatively small differences in yield between the high and low seed rates illustrate the considerable extent to which wheat can withstand or compensate for insect attack.

By varying tilth, cultivation and seed rate, larval infestations were produced differing by a factor of 10 or more, and the results indicate the extent to which wheat-bulb fly infestations can be controlled by cultural means. The effect of seed rate is particularly interesting for it is common practice to increase the seed rate when insect damage is expected. With wheat-bulb fly, the experimental results show clearly that, with an early sown crop, the main effect of a high seed rate is to favour the survival of the pest. Not all the experimental treatments used to produce low infestations could be achieved, or even recommended, in practice. A smooth tilth encourages weed seeds to germinate and so necessitates more cultivation to control them and seed rates of $\frac{2}{3}$ or 1 bus./acre may be too low for general practice. However, by making a smooth tilth, by avoiding cultivation during the oviposition period whenever possible and by drilling early, at the lowest seed rate consistent with high yield, wheat-bulb fly infestations could be reduced greatly.

I am greatly indebted to Mr Cooper of Hill Farm, Gestingthorpe, and Mr Yates of Shore Hall, Cornish Hall End, for providing facilities for the experiments on their farms and to their farm staff who did all the cultural operations. Dr H. E. Gough and Mr F. E. Maskell helped to obtain these facilities and co-operation for me. Several members of the Entomology Department assisted with the experiments, in particular J. R. Lofty and H. Franklin. I am also indebted to Mr J. H. A. Dunwoody who helped to analyse the results and to Mr M. J. R. Healy for advice on their presentation.

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OSCINELLA FRIT L. IN SOUTH WALES. THE RELATIONSHIP BETWEEN SOWING DATE, PLANT DEVELOPMENT AND PROGRESS OF ATTACK IN SPRING OATS

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(With 6 Text-figures)

The degree of lateness of sowing of spring oats in south Wales is reflected in a proportional increase of frit-fly damage to the shoots. Late-sown crops are also more subject to heavy attacks on grain than those sown earlier.

Equations are given relating the development of frit-fly attack both to the growth of the plant and to the date of sowing.

Introduction

Oscinella frit L. is a well-known pest of oats in Great Britain and it frequently reaches epidemic proportions in south Wales. Indeed, in the Usk and Wye Valleys it has been largely responsible for the abandonment of spring in favour of winter varieties of oats.

In Great Britain the major study of the problem, besides Roebuck's (1920) work on the blindness of oats, was that of Cunliffe (1921, 1925, 1928). Cunliffe with Fryer & Gibson (1925) showed that the growth stage of the growing plant determined whether or not it would be attacked. The emphasis in this paper was on the attack of the main shoot, since it was thought that the main panicle-bearing stem was derived from the primary shoot (1928), although in 1925 Cunliffe demonstrated that certain varieties could make up for the loss of primary shoot by panicles derived from subsidiary tillers. However, it was not until 1956 that Labanauskas and Dungan showed the value of these panicle-bearing tillers.

Up to 1952 (Thomas, 1958) the main objective in work on the control of frit fly was a resistant variety. Cultural methods, however, as early as 1889, were regarded as of great importance. Prof. McCracken, in a letter to Miss Ormerod (1890), stated that early sowing and a top dressing of nitrogen was a good method of avoiding attack. Collins (1918) also supported this view, and Petherbridge (1924) showed that early sowing gave a marked increase in yield, with less eggs laid by the frit fly. The work of Cunliffe, Fryer & Gibson (1925) placed these observations on a firmer basis, by showing that the size of the oat plant itself was the key factor in the date of sowing relationship. But in 1921 and again in 1928 Cunliffe showed that an excess of nitrogen did not influence the degree of shoot infestation.

The first trial, statistically arranged, showing the effect of the date of sowing upon

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frit attack, is that of Sheals (1950). This experiment, designed to demonstrate the effect of different manurial treatments on the degree of blindness of oat panicles, showed that there was a significant increase in shoots attacked by *Oscinella frit* as the date of sowing was delayed from 14 April to 12 May, but there was no response to nitrogenous fertilizers.

Methods of investigation

To simulate field conditions as closely as possible, observations were made on large trials, which varied slightly from year to year, but in general were of randomized-block layout, each plot being about 90 yards long and one drill (12–13 rows) wide. This allowed estimation of yields by combine-harvester. In 1953 and 1954 the variety S. 84 was sown at three dates each separated by an interval of 2–3 weeks (see Fig. 5). Each of three sowing dates was replicated six times. In 1955 and 1956, the variables were increased by sowing three varieties, Sun II, Eagle and S. 84. S. 84 was replaced by S. 221 in 1956. In addition, during these 2 years, plots were treated with two levels of nitrogen (0 and 1 cwt. Nitro-Chalk/acre) when drilling. This gave eighteen treatments and these were repeated in two blocks, nitrogen and variety being randomized within each date sub-block. During 1953–5, trials were laid down at both Usk, Monmouthshire, and Wenvoe, Glamorganshire, but in 1956–8 at Usk only. All trials were sown at 4 bus./acre, which produces a stand of about thirty plants per foot length of row.

By 1957 it was clear that neither the varieties available nor application of nitrogen had any significant effect on frit-fly attack when compared with the effects of sowing date. A rather different trial was therefore laid down. This was partly designed to investigate methods of chemical control which have been reported elsewhere (Fidler, 1958; Thomas, 1958) to give information not available in the previous date-of-sowing trials. Thus two adjoining trials were laid down at Usk, one of S. 84 and the other of S. 221 both with plots sown at two different dates, 10 and 30 April. Some plots on both trials were sprayed with an insecticide to obviate the effects of the frit-fly attack, while others remained unsprayed. A complete kill of the pest was not obtained but with data obtained on similar trials (Strickland, 1958) it is possible to relate the effects of pest damage to loss in yield. These 1957 trials were of smaller plots (20 yards by one drill width) to facilitate hand spraying and were therefore harvested and threshed by hand. All yields were corrected to 15% moisture content and expressed as cwt. per acre. In 1958 two further chemical-control trials were laid down at Usk, on Sun II and Eagle sown on 11 April. On these trials new techniques were available for recovering eggs from the soil (Webley, 1957) and for observing fly populations in the field by means of sticky traps. The trap used differed slightly from that described by Ibbotson (1958), being 6 in. square and yellow in colour. It was changed once a week, and kept level with the top of the crop.

In 1953 counts in the field were made of the number of 'dead-hearts' per foot of row; fifty such counts being made in each plot. In addition, some ten attacked plants per plot were collected and dissected in the laboratory to confirm that

Oscinella frit was the species causing the damage. Experience with the latter method indicated that it was somewhat more sensitive in detecting the presence of frit-fly larvae than was the field count of 'dead-hearts' (Light & Strickland, 1959). Therefore damage estimates were standardized in 1954–5 at fifty plants per plot, collected at random in a series of diagonal transverses. Plants were placed in polythene bags and measured in the laboratory from root base to leaf tip. The number of leaves and tillers was counted and the plants then dissected; the number of main shoots and tillers showing obvious symptoms of tunnelling by frit-fly larvae was recorded, as well as the number of larvae and puparia found. In 1956 frit-fly attack was extremely heavy and laboratory examination of fifty plants with as many as ten tillers each proved very laborious. A check was therefore made and it was found that by taking a sample of only twenty-five plants per plot, the within-plot standard error would only be increased from ± 11 to $\pm 15\%$. Recent figures published by Strickland (1959) suggest that these errors may be even lower than this. This smaller sample was thereafter adopted as standard practice.

Such plant sampling was done at weekly intervals from the time when it was estimated that germination was completed, until the plant had reached about the $5\frac{1}{2}$-leaf stage. A plant count was also taken when germination was complete. In no case during these trials was there any significant reduction in the number of germinating plants, which could be assigned to frit-fly attack. This seemed to confirm the assertion of Cunliffe *et al.* (1925) that the oat plant is highly resistant to frit fly up to the 1st-leaf stage. Subsequent work on seed dressings and egg-laying habits during 1957 and 1958 seems to suggest that there may be considerable losses in plants at this early stage, but it is not easy to prove that this is entirely due to frit-fly attack.

Observations on second-generation attack by frit fly on oat grains in the developing panicle were mainly confined to the standard methods of analysis. In this 100 heads are taken at random from each plot just previous to harvesting. When dry the grain is rubbed out, thoroughly mixed, coned, quartered and 100 main and 100 bosom grains selected at random. These are dissected and the number of damaged grains and puparia present recorded. The proportion of main to bosom grains was at no time found to differ significantly from the 1 : 1 ratio. Other observations made included the number of spikelets per head at various dates and their relation to earlier frit-fly attacks and the number of heads per foot in relation to tiller development and plant loss under a high level of attack.

Plant development during shoot attack

Cunliffe (1925) studied the effects of mechanically killing the primary shoot plus one or more tillers. He found that these were replaced by tillers, but resulting panicles were reduced in size. Frey & Wiggans (1957) found that some varieties were better than others in the capacity to form tillers, but that the main factor was the effect of spacing. Thus if a shoot is killed, e.g. by frit-fly attack, the same plant or an adjoining one will replace that shoot by developing extra tillers. However,

if the attack comes late the plant may have passed the stage at which it is still capable of producing tillers, as is illustrated in Fig. 1. This shows the characteristics of four types of crops as found in the present series of trials, and each is a composite graph showing the number of leaves and tillers per plant and the percentage frit-fly attack on the primary shoot and tillers. Although these four crops are not in order of sowing, being from different years, they are in order of severity and of increasing earliness of attack as compared with plant development.

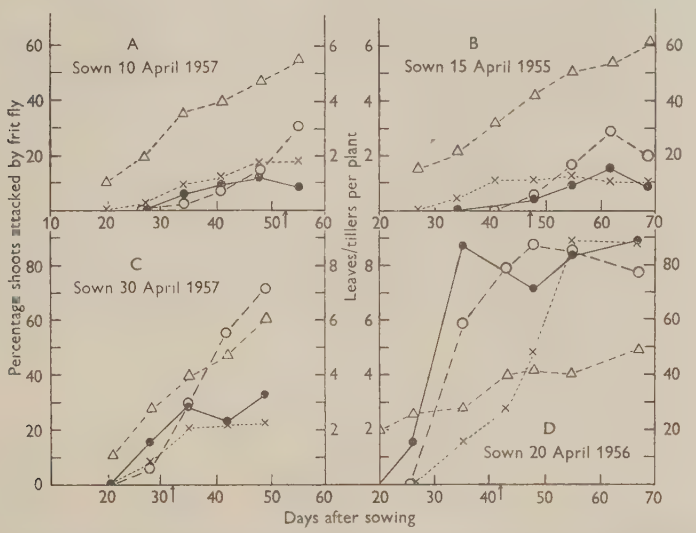

Fig. 1. The development of the plant and frit-fly attack in four trial oat crops. ↑, 1 June; --△--, leaves per plant; --×--, tillers per plant; —●—, main shoot attack; --○--, tiller attack.

These weekly figures for attacked shoots are aggregates of the shoots attacked in previous weeks up to the time of sampling. After 1953 all observations were made weekly by dissection of samples in the laboratory and, at least in the early stages, the primary shoot could be distinguished from subsequently formed tillers. This is not, however, the case in later stages, since it is not generally possible to state that a particular panicle is formed from a primary shoot, because if this is killed in an early stage it later shrivels up and disappears. In all cases the attack on the stem reached a maximum about the 4th-leaf stage, but the attack on the tillers, especially when the attack was heavy, and the crop sown relatively early, continued after this stage (Fig. 1 A). When observations were carried on beyond this stage it was always found that the numbers of attacked shoots decreased, due to the older 'dead-hearts' falling out (Fig. 1 B). Generally the maximum number of tillers was reached between the 3rd- and 4th-leaf stage, but if the frit-fly attack took place before this stage was completed, further tillers were produced. In Fig. 1 C the attack was late

and the plants had passed the stage when tillers could still be formed. In 1956 (Fig. 1 D) the attack was at a very high level and as new tillers were formed they in turn were attacked. The plants were held in the stage when tillers could continue to be formed and before the attack was completed an average of nearly nine tillers per plant was produced. Of these over 80% had been attacked. This left the plants with more than the usual number of healthy tillers, but a count of plants at harvest time showed that only 38·8% of the plants which had germinated had survived to that stage. Spacing had therefore been greater than normal and the surviving plants had produced extra shoots to compensate for this.

In 1955 the effects were studied of an application of nitrogen on the tillering of healthy and attacked plants. For this, samples of 100 healthy and 100 attacked plants were abstracted at random from the field samples and their tillering capacity analysed (Table 1). It was found that both nitrogen and frit-fly attack gave a significant increase in the number of tillers formed. The effects of sowing date were, however, more complicated. In the healthy plants, on postponing the sowing, there was a marked decrease in the number of tillers formed. This was not the case in the damaged plants, because the attack was much heavier in the later sowings, thus increasing the number of tillers. Without nitrogen there were in fact more tillers in the last sowing, while where nitrogen had been applied the tillering was about even in all sowings.

TABLE 1. *Effects of nitrogen (tillers per plant)*

(Mean of 100, 1955 trial.)

	Healthy plants		Attacked plants	
	0 nitrogen	+ nitrogen	0 nitrogen	+ nitrogen
First sowing	0·70	1·86	2·04	2·50
Second sowing	0·52	1·06	1·65	2·26
Third sowing	0·25	0·33	2·31	2·54

Least significant difference = 0·185 ($P = 0·05$).

Analysis of attack development

Weekly records of frit-fly attack and plant development, etc., were available from some twenty trials of oats sown in south Wales between 15 March and 4 May 1954–8. The development of the frit-fly attack on the shoots, expressed as a percentage, was plotted for each crop against the number of leaves on the main stem. This gave a more satisfactory result than plotting against time, since, if an unusually cold or warm spell occurs during this period, any check or acceleration it causes in frit-fly damage is also reflected in the development of the oat plant. A typical set of such curves is shown in Fig. 2 from the trial at Usk in 1955. This demonstrates that the increase in attack is gradual at first from about the 2nd-leaf stage, becomes more rapid and then slackens towards a maximum about the 5th-leaf stage.

After the maximum is reached, if observations are continued, the percentage attack usually falls to a lower figure. In 1954 counts were made by observing damage in the field over some 60 ft. of row per plot, picked at random in 1 ft.

lengths. Both total and damaged shoots were counted. In the third sowing where this final decrease was most marked, it was found that, the week after the peak was observed, there was a fall of 19·4% in the number of attacked shoots, but only 9·4% in the healthy ones. Thus the proportion of attacked shoots to the total decreases. It might be thought that this was due to the death of the smaller plants being crowded out by their more healthy neighbours, but in later years counts were made by collecting samples of twenty-five plants per plot and examining them in the laboratory. This same fall in attack was noted and is due to the plant losing some shoots. This takes place soon after the 6th-leaf stage; but the panicle usually emerges from the 7th-leaf sheath, so that at this stage the plant probably discards some small shoots, especially if they are moribund and unlikely to form a panicle at a later date.

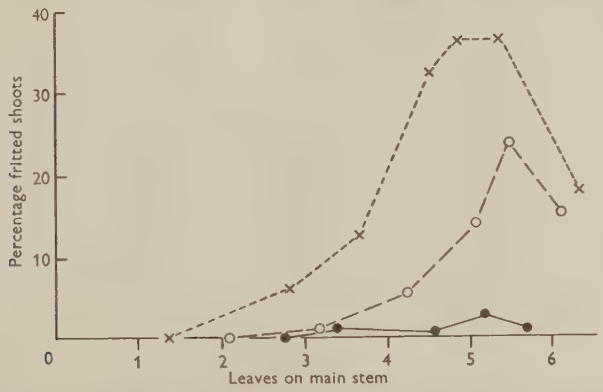

Fig. 2. The development of frit-fly attack in relation to plant development. *Key*. Sown 31 March, —●—; sown 15 April, --○--; sown 30 April, --×--.

Inspection of these development curves indicated that, ignoring the declining part of the curve, they were approximately sigmoid. Probit transformation was therefore applied to the weekly damage counts, expressed as a proportion of the respective peak value. A probit value of 8·8 was taken to represent this latter figure, since it is equivalent to 99·9% damage. The increase to the full probit of 10 is not likely to be detectable in a field count.

Preliminary plotting showed that with higher degrees of attack the curves gave a good approximation to a straight line, but at low levels the points tended to be more scattered.

In order to find the best straight line to fit the observed data transformed to probits, a regression equation was calculated for each crop. Some twenty regression lines were then plotted and labelled with their respective dates of sowing. From these it was seen that both the stage at which attack theoretically started, and the rate of increase, appeared to vary with the sowing date. These are listed in Table 2. There was no evidence, however, of a direct relationship between the stage at which the

peak damage was attained and the date of sowing. This is the relationship in which the farmer is most interested and so that it could be calculated approximately, the correlations were calculated between rate of increase of attack and the leaf stage at which it theoretically starts with sowing date respectively.

$$G = 5{\cdot}01 - 0{\cdot}0499 \pm 0{\cdot}00626 \times T, \tag{1}$$

where G is the gradient of the probit curve, T is the time of sowing of the crop stated in days after 1 March; $r = 0{\cdot}511$ with 18 D.F.

$$c = 4{\cdot}92 - 0{\cdot}0698 \pm 0{\cdot}0144 \times T, \tag{2}$$

where c = the leaf stage when damage theoretically starts; $r = 0{\cdot}752$ with 18 D.F.

TABLE 2. *Probit analysis of attack development*

Sowing date	Maximum attack (%)	G	S.E.	c
19 March	9·1	4·794	±0·519	3·058
31	4·1	2·929	±1·068	2·347
31	1·4	—	—	4·000
1 April	4·7	2·769	±0·782	2·369
4	33·1	5·850	±0·095	2·766
6	5·7	2·800	±0·690	1·771
10	23·0	2·269	±0·479	1·873
10	11·5	1·738	±0·837	—
11	44·0	2·475	±1·187	1·842
11	45·5	1·575	±0·906	1·060
12	9·2	3·552	±0·707	2·266
15	23·3	3·508	±0·721	2·939
15	23·5	2·208	±1·030	2·330
15	19·0	2·971	±1·127	2·677
20	12·9	3·699	±0·412	1·217
20	86·2	1·922	±0·374	0·210
23	22·6	3·200	±0·265	1·262
30	60·0	1·654	±0·547	0·870
30	33·0	1·676	±0·686	0·471
30	42·0	1·715	±1·082	0·853
30	31·9	2·936	±1·013	—
4 May	14·8	—	—	−0·0255

The probit lines calculated from these equations for sowing dates of 15 March and 4 May have been plotted in Fig. 3. The leaf stage, at which each of the twenty curves reach the arbitrary peak probit of 8·8, was then calculated and this gave a mean value of $5{\cdot}172 \pm 0{\cdot}195$ leaves.

From the original field observations the estimated maxima were also recorded and these gave a mean of $5{\cdot}2 \pm 0{\cdot}22$ leaves.

Using the symbols defined above a general equation for the probit development curve can be expressed as $P = G\,(L - c)$ where P is the probit proportion of the final attack and L the leaf stage found when sampling. Both G and c are functions of the date of sowing T and can therefore be substituted in this equation giving

$$P = L\,(5{\cdot}01 - 0{\cdot}0499\,T) + T\,(0{\cdot}759 - 0{\cdot}00348\,T) - 24{\cdot}67. \tag{3}$$

This can be solved for any given leaf stage found at the time of sampling a crop, and from the percentage damage found an estimate of the final attack can be obtained.

On the right-hand side of Fig. 3 have been marked the percentage values of the final attack equivalent to the probits on the left side. Because of the variation in the size of the plant at the final attack, it would be unsafe to extrapolate from any point of less than one-third, or better one-half, of the final value. Such values are not reached until at least after the $3\frac{1}{2}$-leaf stage so that no great warning of final damage can be obtained. However, such results might well be useful when advising the use of a late dieldrin spray (Fidler, 1958), for it could be determined, at least roughly, how much more damage might be expected and whether the prevention of this was economically justified.

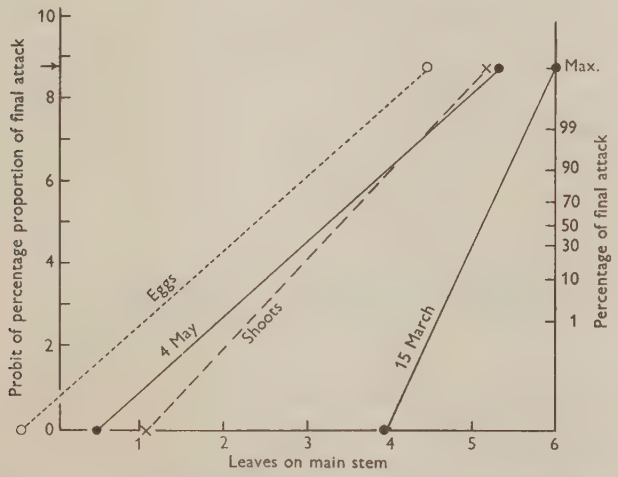

Fig. 3. Theoretical rate of frit-fly attack in relation to plant development at early and late sowing dates; with mean egg and shoot attack development in the 1956–8 trials.

Relationship with egg laying

Counts of eggs at weekly intervals with the Cardiff egg sampler started in 1956 and figures are now available from five of the trials. The form of the development curve is very similar to that of the shoots, and probit analysis was therefore again applied. Results are shown in Table 3 comparing the coefficients calculated for both shoot attack and egg laying for each of the five crops. Generally the gradient of the egg curve is rather less than that for the shoot attack, but with the few figures at present available the difference in means cannot be considered significant. However, this difference might be expected, since the egg is likely to take a decreasing time to develop and form a larva attacking a shoot as the season progresses. The

main exception to this was the 1956 figure, but in this year the technique was only in a developmental stage and the figure is probably not very accurate. From the means of these five drops two curves have been plotted in Fig. 3.

From these five probit curves it is also possible to estimate the average time elapsing between egg laying and the laboratory recording of the dissected shoot as attacked. This is most critical at the probit value of 5, i.e. when half the maximum attack has been reached. The leaf stages for both eggs and shoots, as well as the differences, are recorded in Table 3. Although the figures vary somewhat from crop to crop the difference between the means, as shown by an analysis of variance, is $1{\cdot}22 \pm 0{\cdot}56$ and is significant at $P = 0{\cdot}02$. There is some suggestion that the difference may vary with the sowing date, the later crops having a shorter time between egg count and attacked shoot, but there are at present insufficient data to establish this.

TABLE 3. *Relationship between egg counts and shoot attack*

	Development coefficient		Leaf stage at half attack (probit = 5)			Eggs/ five grabs	% shoot attack	Ratio egg/ shoot
Sowing date	Eggs	Shoots	Eggs	Shoots	Difference			
20. iv. 56	2·127	1·922	2·36	2·81	0·45	514	86·2	5·96
10. iv. 57	1·740	1·738	2·28	3·80	1·52	102·5	18·0	5·69
30. iv. 57	2·106	2·936	2·05	2·68	0·63	139·5	32·9	4·24
11. iv. 58 (Sun II)	1·538	1·575	2·40	4·23	1·83	31·0	60·0	0·52
11. iv. 58 (Eagle)	1·460	2·475	2·62	4·27	1·65	29·0	44·0	0·66
Means	1·794	2·129	2·34	3·56	1·22			

Since the peak shoot attack is reached at about 5·2 leaves, it is apparent from these figures that no more eggs are laid on the plant after the 4th-leaf stage. This confirms the statement made by Cunliffe *et al.* (1925).

Also from the records of these five crops a comparison has been made between the peak shoot attack expressed as a percentage and the maximum number of eggs recorded per five grabs with the Cardiff sampler. This gives figures of much the same order for the crops sown in 1956 and 1957, but the two figures for 1958 are considerably lower. However, when further data of this type are available, it may be possible to forecast a shoot attack from information gained by earlier egg sampling.

Leaf development and sowing date

In these studies, development of the oat plants has been measured as the number of leaves on the main stem, expressed as the mean of a field sample of twenty-five plants. From the twenty crops on which full weekly data are available it is possible to examine the rate of growth as affected by different times of sowing. From each crop development curve the date was estimated at which each leaf stage was reached. This gave a number of dates for each leaf stage, 2–5, with a corresponding sowing date from which regressions could then be calculated. The estimated coefficients

with their errors are given in Table 4 and the curves plotted in Fig. 4. Although the difference between any two adjoining coefficients is not significant, there is a steady decrease as sowing date progresses. This is probably real, since it means that later in the season less time is taken to grow from one stage to the next, probably owing to increasing temperature and day length. What is less easy to explain is that for a given sowing date each leaf stage takes longer to develop than its predecessor.

TABLE 4. *Leaf development and sowing date*

Leaf stage	Coefficient	Error
2nd	0·781	±0·159
3rd	0·774	±0·075
4th	0·740	±0·124
5th	0·712	±0·202

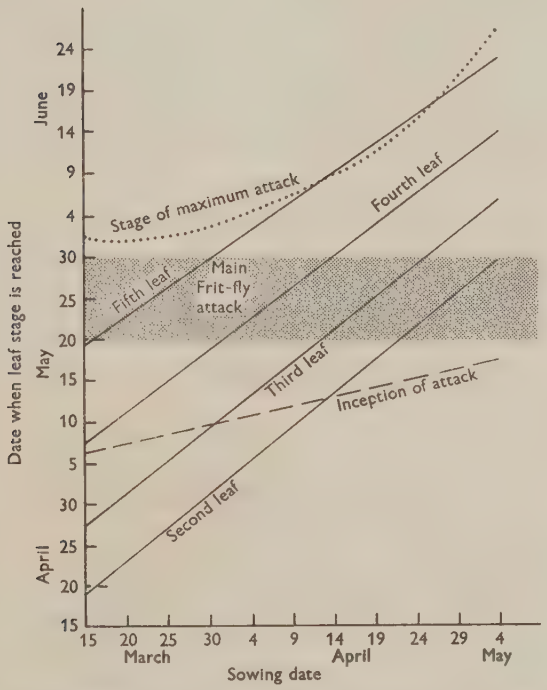

Fig. 4. Mean date of leaf formation in south Wales in relation to sowing date of crop; with dates of first and maximum attack by frit fly.

It is possible to calculate from equation (2), given above, the leaf stage at which damage is expected first to take place in crops sown at various dates. From the data given in Fig. 4 these leaf stages can be converted to actual dates and these have been entered on the graph.

No simple relationship was found between the leaf stage when peak attack is reached and time of sowing, but from the general equation (3) taking $P = 8{\cdot}8$ this can be calculated:

$$L_{\text{peak}} = \frac{8{\cdot}8}{G} + c, \tag{4}$$

where both G and c are known in terms of T, the date of sowing. These leaf stages were also converted to dates and entered on Fig. 4.

The main period of frit-fly activity can be taken as 20–31 May as suggested by Jepson & Southwood (1958) and Cunliffe (1928) and this fits well with our observations made in south Wales during the past 6 years. Fig. 4 shows that with crops sown on 15 March, although frit-fly damage theoretically starts on 6 May, the crop will have reached just over five leaves by the period of main activity and be resistant to further attack. Field observations have shown (Table 8) that crops sown before this date are unlikely to sustain an appreciable attack. However, records indicate that some damage to such crops continues until the end of May, but this is confined to the few small tillers produced at this late stage. Crops sown on 4 May will have emerged from the soil only the previous week when first damage is expected and this will be at a time when full frit-fly activity is to be found in the field.

Plant development after shoot attack

Bonnett (1937) has shown that the formation of the panicle in the oat plant starts at a very early stage. If this growing point in the primary shoot is killed by frit fly its function can to some extent be served by later-formed tillers. This was studied in detail in the 1956 trials where the attack, especially in the last sowing, was extremely heavy. Table 5 gives the main factors for each of the three sowing dates.

Table 5. *Effects of attack on tiller and panicle formation, Usk,* 1956

Factor	Date crop sown		
	19 March	4 April	20 April
Main shoot attack (%)	15·5	32·9	68·4
Tiller attack (%)	9·0	32·8	87·0
Plant survival (%)	81·5	83·0	38·3
Tillers per plant	1·74	1·65	8·94
Panicles per plant	1·14	1·37	2·98
Panicles formed per 100 healthy shoots	18·7	87·0	213
Spikelets per head	26·12	22·58	12·10

It is apparent that the number of tillers formed is in direct relation to the degree of attack and in inverse relation to plant survival. On the other hand, the actual number of heads produced per plant was only about $2\frac{1}{2}$ times as great in the last sowing as in the other two, although many more tillers had been formed. A calculation was made of the number of healthy tillers remaining when final counts were made at the end of the frit-fly attack on the shoots, compared with the ultimate

number of panicles formed. It was found that in the first sowing only 18·7 out of every 100 shoots formed panicles, while in the second sowing the figure was 87·0. In the third sowing it was found that on 26 June there was only just over one healthy tiller per plant, but more tillers must have been found after this date, when attack had ceased, with the result that there was more than twice this number of heads present at harvest time.

In addition to this increase in number of panicles per plant there was a marked decrease in the number of spikelets per head, there being less than half the number in the third sowing as compared with the first. This study was taken further in 1958 when heads were examined on a plot which had sustained an attack of 32% in the main shoots and 42·6% in the tillers. This showed a mean of 4·88 tillers per plant. The crop was sown on 11 April and the first panicles started to emerge on 1 July. Each week twelve panicles which were just emerging from their sheaths were marked with a dated label. At the same time a further ten panicles were collected and the eggs washed off and collected. This method does not, however, give an absolute return of the numbers laid on the spikelets, since the numbers recovered only represent about half the puparia subsequently found. Sampling has shown that eggs are only laid on the spikelet up to the time of anthesis, so all the eggs must have been laid during the first week after the emergence of the head from the sheath.

TABLE 6. *Dated panicles, Usk Institute,* 1958

(Ten to twelve newly emerging panicles labelled each week and collected 22 August.)

Date labelled	Adult on trap	Eggs/ 100 spikelets*	Full spikelets	Puparia in grain	Flies emerged after 22. viii. 58
1 July	17	49	541	192	13
9 July	139	33	441	136	74
15 July	90	96	268	49	31
22 July	144	147	218	20	15
29 July	29	42	105	16	12
5 August	4	49	130	8	7
15 August	5	54	51	0	0

* From ten similar panicles collected and washed out each week.

Table 6 shows that the first panicles were by far the largest and that subsequently the number of spikelets gradually decreased, week by week. It was not possible at this stage of growth to say whether these first panicles were formed by primary shoots or by tillers, but it seems more than likely that the small late heads were the product of tillers formed after the shoot attack had taken place. In connexion with this Labanauskas & Dungan (1956) suggest that although panicles formed by the main stem are the largest, the aggregate of those formed by the tillers may be even greater. The greatest grain attack took place on the heads emerging during the first 2 weeks in spite of the fact that adult activity, as shown by a sticky trap in the crop, was most in the second to fourth weeks. The last panicles to emerge were not attacked at all, although flies were still on the wing.

On 22 August the labelled panicles were collected and placed in polythene bags

and the number of flies emerging counted. It is clear that the collecting date was rather too late and many flies had already escaped from the first two batches of labelled heads.

EFFECTS OF SOWING DATE ON FRIT-FLY ATTACK

(*a*) *Attack on the shoots*

In addition to the more detailed studies on individual plants described above observations were at the same time made of the oat crop as a whole. For peak of attack of the first generation the stage between the 5th and 6th leaf was taken as the

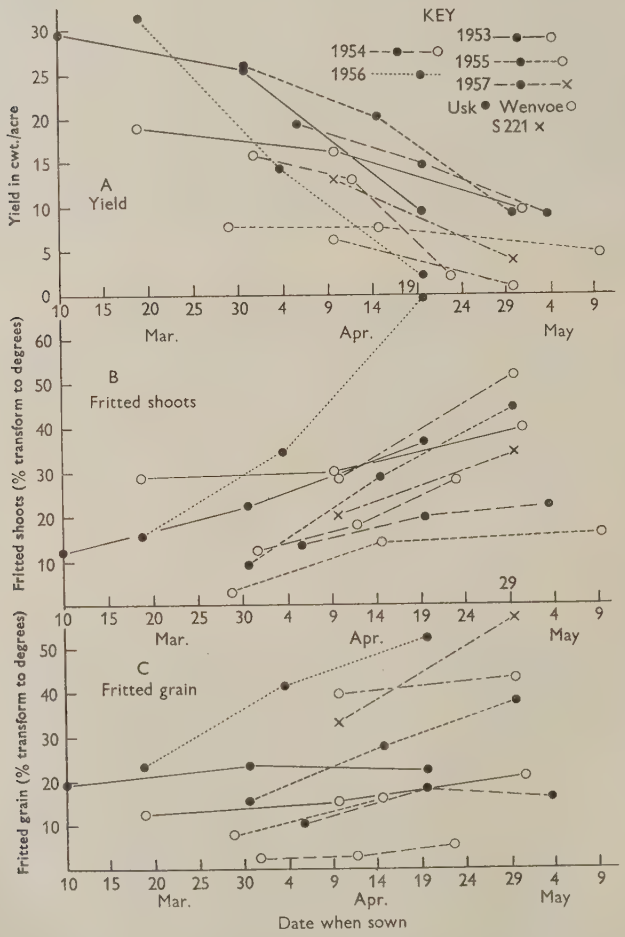

Fig. 5. The relationship between sowing date, yield and attacks by frit fly in various trials in south Wales.

maximum, and after striking percentages the results were subjected to inverse sine transformation to make them additive. These results were plotted in Fig. 5 B against sowing date for each of the individual trials.

It is apparent that the steepness of the slope of any particular curve is some measure of the severity of the attack at that particular site, but it must not be assumed that these curves continue to slope upwards beyond the period studied. The latest crop sown in this series was on 10 May in a year (1955) when frit-fly attack was both late and slight, and there is some indication of a fall off in the attack. Thomas (1954) quotes figures from a number of crops sown in the middle of May in which there was a lower attack than in the April-sown crops. Such late crops are not, however, frequent in commercial practice in south Wales.

Even the earliest sown crops in all these trials had some attack by this pest. It is known from field observations that, before the main body of frit-fly attack, there is often some damage apparent but this is usually slight. For practical purposes therefore in most years the early part of this can be assumed to be a straight line and to obtain the best fit, regressions were calculated for each trial, the coefficients with their standard errors being listed in Table 7. No great error is likely therefore if the curves are extrapolated backwards to zero, giving the latest date on which a crop can be sown and be free from first-generation frit attack in this area. These have been calculated from the various regressions and the dates are listed in Table 8. In most years this date varies by a few days round about 15 March according to whether the frit fly was early or late in its first emergence. In 1953, however, this date was very much earlier, possibly due to the 'pre-main' attack being large in comparison with the main attack. Thus it is suggested that a crop sown as early as 15 February at Usk would have sustained some attack, while at Wenvoe even winter-sown oats would have been attacked. This confirms field observations that in exceptional years even very early crops sown in February suffer some attack.

TABLE 7. *Regressions of fritted shoots on sowing dates*

Site and year	Fritted shoots		D.F.	Index (*I*)
	Coef.	S.E.		
1953 Usk	0·593	±0·075	17	32
Wenvoe	0·249	±0·066	14	32
1954 Usk	0·309	±0·063	17	9
Wenvoe	0·729	±0·121	17	22
1955 Usk	1·016	±0·077	35	31
Wenvoe	0·308	±0·027	35	9
1956 Usk	1·675	±0·135	35	48
1957 S. 221	0·734	±0·111	7	22
S. 84	1·176	±0·187	5	34

Making certain assumptions, it is possible to calculate a rough index (*I*) as a measure of first-generation frit-fly attack for each site and year. Thus the average sowing date before which no attack is to be expected lies within a day or so of 15 March, if 1953 is regarded as exceptional and not included. An arbitrary date is

then selected, such as 14 April, which as shown below is the mode of sowing dates of commercial spring oat crops in south Wales. This is 30 days after 15 March and the fritted shoot coefficients given in Table 7 are then multiplied by this factor, giving the index (I) as shown in the last column of the table. The indices for 1953 have been abstracted from Fig. 5B, since in such exceptional years adjustments have to be made for the earlier primary dates.

TABLE 8. *Latest sowing date on which no shoot attack occurred*

1953	Usk	15 February
	Wenvoe	Before Christmas
1954	Usk	9 March
	Wenvoe	15 March
1955	Usk	20 March
	Wenvoe	12 March
1956	Usk	10 March
1957	S. 221	13 March
	S. 84	16 March

These indices vary not only from year to year, but also between sites in the same year. Unfortunately, there is at present insufficient knowledge to account for these variations, but the mean of a number of such indices from various sites should give a good indication of the average attack in the district for any given year.

(*b*) *Attack on the grain*

Frit flies hatching out from the shoots lay their eggs on the emerging panicles. If the incidence of shoot attack is high, it can be assumed, other factors such as immigration of flies into the field being equal, that there will be a large number of flies available to attack the grain (Jepson & Southwood, 1958). Thus early-sown fields will tend to have a lower population of flies at panicle emergence than would late-sown crops in the same year. However, in the present series, trial plots, sown at three dates, were randomized within each block and therefore flies hatching from plants sown at all three dates would tend to intermingle over all plots. Panicles emerging from plants sown at different dates would therefore be subjected to egg laying over a much longer period than they would in a field, the whole of which was sown at one date. Any differences found in grain attack in these trials must therefore be due to difference in susceptibility of the panicles to frit-fly attack.

That such differences are real is shown in Fig. 5C where the percentage (transformed to degrees) attacked grain is plotted against the date when the crop was sown for each trial. These percentages were derived as a mean of the main and bosom-grain figures. It is found in practice (Empson, 1957) that the bosom grain is generally attacked less than the main grain, but since both are present in the panicle in substantially equal numbers, a mean of the two represents the actual number of grains lost by the farmer. These curves are very similar in form to those of shoot attack, plotted in Fig. 5B, but the upward slope on delayed sowing is less

acute than with the spring generation. A high incidence of shoot attack, e.g. in 1956, tends to give a large grain attack although this is not always so, e.g. 1953 when the spring attack was exceptionally early. That there is some overall connexion is shown by calculating the regression between shoot and grain attack, for each plot

$$g = 5{\cdot}50 + 0{\cdot}679 \pm 0{\cdot}0404\, t, \qquad (5)$$

where g is the grain attack and t is the shoot attack, both expressed as the angular value of the percentage, $r = 0{\cdot}767$. This regression, with 199 D.F., is very highly significant.

A rather better picture of crops under normal field conditions is shown by the 'Extensive Survey' carried out by the N.A.A.S. in south Wales each year, with the exception of 1951, from 1946–57. 'Grab samples' of 100 heads were taken from

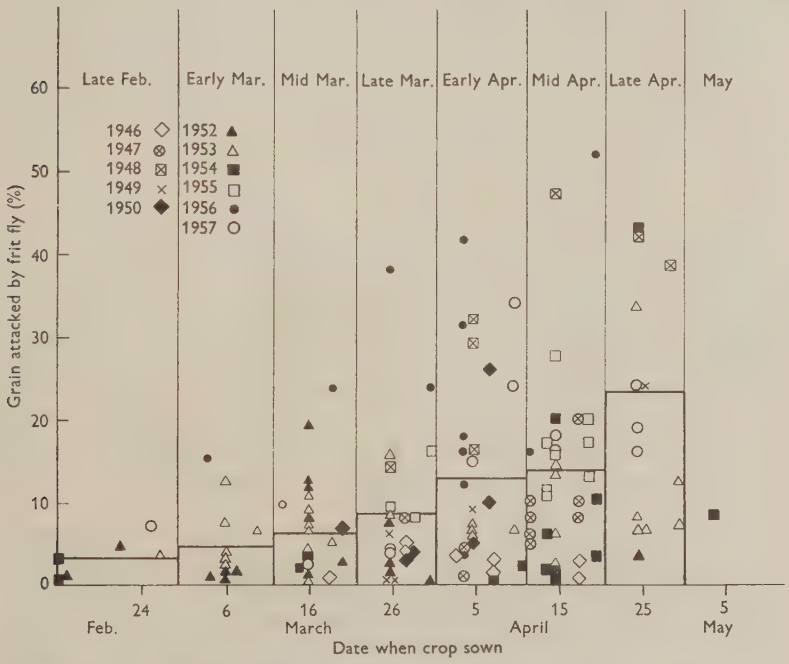

Fig. 6. Scatter graph showing grain attack by frit fly in relation to sowing date on field crops in south Wales.

about twelve spring oat fields, selected at random, each year and examined for frit damage in the grains. Date of sowing was noted. Thomas (1954) studied similar figures for 1947–51 for the whole of England and Wales but was unable to find very clear indication of a relation with sowing date, possibly owing to the wide variation of conditions over the area studied. Thus it is suggested (Strickland, 1958)

that attack on the grain is almost entirely absent in the northern counties of England. The 135 results from south Wales are plotted as a scatter graph in Fig. 6. It is apparent that the later-sown crops are more liable to high grain attack than early crops. Sowing dates have been divided into the classes: late February, early, mid- and late March, early, mid- and late April and early May. The mean grain attacks found in crops of each of these periods have been plotted as a superimposed histogram, clearly indicating that this relation holds true in a field crop of oats.

The mean date of sowing as calculated from all the 135 observations works out at 3 April. This does not include the large number of winter-sown oat crops which are grown most years in south Wales. This date is 10 days earlier than that for the mean of the crops sown in the trials carried out by us, and indicates that at least a proportion of the farm crops in most years would have entirely escaped frit-fly damage in the shoot stage, although all except five had some degree of grain damage. This mean sowing date of 3 April is somewhat misleading in that the scatter graph is highly skew. A better indication is given calculating the mode which falls on 14 April and by considering that 45% of the crops were sown during the period early to mid-April. The mean frit-fly grain attack over all the crops examined in this 'Extensive Survey' in south Wales was 11·8%.

Effect of frit-fly attack and sowing date on yields

It is generally accepted that in the absence of frit-fly attacks, the shorter the time available for development between sowing and harvesting, the smaller the crop of spring oats. Unfortunately, in none of the trials carried out in south Wales was there, at any sowing date, a complete absence of damage by frit fly, so it was impossible to assess directly the loss in crop caused simply by delay in sowing. The overall mean yields for each site at each sowing date have been plotted in Fig. 5A and thus include the effects of both the above factors. Where the influence of the varieties and an application of nitrogen were included in the trials (1955, 1956) the results have been bulked and differences taken out with the error variance where appropriate.

Each curve shows a fall in yield with delayed sowing, but the rate of fall differs from year to year. The general size of the yield differs between the two sites, tending to be higher at Usk than at Wenvoe, but in any one year the rate of fall tends to be similar on both sites. These site differences are probably due to differences in soil fertility. The rate of fall was least in 1953 and greatest in 1956, the 2 years when frit-fly damage was at its lowest and highest incidence, respectively. Over the period studied, i.e. that most usual for the sowing of commercial crops of spring oats in south Wales, the relation between yield and sowing date tends to be nearly direct. There is, however, in most years a tendency for crops sown after the middle of April to show a slightly more rapid fall in yield than those sown earlier in the year.

This series of curves was plotted on the same basis as that showing the first-generation frit-fly attack in Fig. 5B and inspection shows a very close relationship

between them. Thus a very rapid increase in the incidence of frit-fly larvae in the shoots, e.g. in 1956, resulted in an equally rapid decrease in yield. Regression coefficients were therefore calculated for each of the yield curves and are listed in Table 9. These figures represent the mean loss in crop for each day's delay in sowing and can be compared with the similar set in Table 7 for increase in frit-fly shoot attack. Since there was apparently a close relationship between the two, a further regression was calculated

$$Yd = 0{\cdot}145 + 0{\cdot}397 \pm 0{\cdot}137\ td, \qquad (6)$$

where Yd = the loss in yield in cwt. per acre and td = increase in frit-fly shoot attack, expressed as an angular transformation of the percentage, both for each day's delay in sowing the oat crop. This is significant at $P = 0{\cdot}05$ (D.F. = 8), but it does not necessarily follow from this that frit-fly damage is the cause of this increase in crop loss. It might be argued that this relationship only holds true because both factors are controlled by the same change in weather conditions. Some indirect evidence is, however, available on this point.

TABLE 9. *Yield × sowing date regression coefficients*

Site and year	With frit attack: Coef.	With frit attack: S.E.	Without frit	Difference
1953 Usk	−0·496	±0·039	−0·368	0·128
Wenvoe	−0·213	±0·026	−0·132	0·082
1954 Usk	−0·369	±0·003	−0·264	0·105
Wenvoe	−0·629	±0·027	−0·464	0·165
1955 Usk	−0·564	±0·021	−0·231	0·332
Wenvoe	−0·079	±0·012	+0·200	0·279
1956 Usk	−0·929	±0·056	−0·447	0·482
1957 S. 221	−0·464	±0·054	−0·110	0·354
S. 84	−0·268	±0·019	−0·015	0·253
Mean	−0·446		−0·203	0·242

Least significant difference = 0·103 ($P = 0{\cdot}05$).

A trial of spring oats was shown by the Crop Husbandry Officers of the N.A.A.S. in Pembrokeshire on 13, 22 and 29 April 1957, and no frit-fly damage to either shoot or grain was recorded. These results have been made available by the kindness of Mr I. Prytherch and from them a mean daily loss of 0·301 cwt./acre has been calculated. This can be compared with the mean figure of 0·446 cwt./acre obtained from the figures in Table 9.

As mentioned above, in 1957 some plots on each of the two trials were sprayed with an insecticide, reducing to a varying extent the incidence of frit-fly attack. Assuming that the reduction in yield is, over this range, proportional to the percentage (transformed to angles) frit-fly attacks (Strickland, 1958), it is possible to calculate how large the crop might have been at each sowing date, had all the damage by frit fly been prevented by these treatments. This was done and is shown in Table 10. Thus, in the absence of frit-fly attack, the loss in crop for each day's

delay in sowing would have been 0·36 cwt./acre with the S. 221 trial, and 0·18 cwt./acre for the S. 84 trial.

Strickland (1958) has shown that an estimate of the relationship between crop loss and frit-fly attacks can be obtained by reducing pest density on a plot scale with insecticides. He has published the equation

$$\text{Yield} = 22{\cdot}7 - 0{\cdot}137 \pm 0{\cdot}029\ Xt - 0{\cdot}185 \pm 0{\cdot}032\ Xg \text{ cwt./acre}, \qquad (7)$$

when Xt = angular value of mean percentage tiller attack at growth stage 3–4, i.e. about the 5th-leaf stage. Xg = angular value of mean percentage grain attack at harvest.

TABLE 10. *Calculated loss of crop in sprayed trials* 1957

Variety and sowing date	Sprayed plots: Shoot attack	Sprayed plots: Grain attack	Sprayed plots: Yield	Unsprayed plots: Shoot attack	Unsprayed plots: Grain attack	Unsprayed plots: Yield	Theoretical unfritted yield
S. 221 10 April	8·2	11·9	17·1	30·3	30·8	13·0	18·1
S. 221 30 April	12·5	31·9	8·0	70·2	56·3	3·7	10·9
S. 84 10 April	2·3	41·1	7·1	22·7	46·3	6·3	7·6
S. 84 30 April	18·7	47·2	3·2	61·8	49·3	0·9	4·0

This equation gives coefficients calculated from trials carried out in various parts of England and Wales and therefore rather generalized, but he does give coefficients of 0·190 and 0·184 for the tiller and grain attacks, respectively, obtained from trials in south Wales and these have been used here. Moreover, little is known of the effects on yield of very low frit-fly attacks. Bearing this in mind, the equation can be used to give some indication of the loss in yield caused by the high levels of frit-fly attack recorded in these trials and from this the theoretical yields which might have been found in the absence of this pest.

This was done for each of the nine trials and new regression coefficients calculated, giving a mean loss of crop of 0·203 cwt./acre for each day's delay in sowing, which can be compared with the mean loss, when including frit-fly attacks, of 0·446 (Table 9). This latter figure does not, however, mean a great deal because, as shown above, there are very wide differences between frit-fly attacks found in different years and sites, and therefore in the proportion of yield loss attributable to this factor. However, these two sets of figures have been subjected to an analysis of variance and it can be seen that only at Wenvoe in 1953 was the difference so small as not to be significant at $P = 0{\cdot}05$.

These four results are of much the same order, with the exception of that from the S. 84 trial at Usk in 1957 where the harvesting of the first sowing was delayed in bad weather and when finally cut much of the grain had been shed. The second sowing was cut on the same day and there was therefore less difference between the two yields than might have been found under more normal conditions. It seems probable therefore that the loss in yield caused by each day's delay in sowing is little more than about $\frac{1}{4}$ cwt./acre in the absence of frit-fly attack. The losses attributable to this pest vary greatly from year to year and may be as high as twice this figure, but in normal years in south Wales are probably of about the same order.

The constants found in all the above calculations are only likely to be relevant under climatological conditions similar to those found in south Wales. Moreover, minor variation in rates of plant development, etc., can be caused by planting unusual varieties or applying heavy dressings of nitrogen. However, the general principles outlined in this paper are likely to apply over a wide area and in most seasons. But it would be unwise to extrapolate from these data to crops sown outside the range of dates studied. In south Wales spring oats sown during this period have their susceptible stage during the period of main frit-fly activity; hence the heavy losses suffered by most growers of this crop in the area.

The writers are grateful to Mr S. Jones of Llanover, and Mr Emlyn Williams of Wenvoe, for permission to carry out trials on their farms; also to the Principal and Governors of the Monmouthshire Institute of Agriculture, Usk, for continuous co-operation in this work. Mr I. Prytherch and various District Officers of the N.A.A.S. must be thanked for help in laying out the earlier series of trials in this area.

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THE EFFECT OF GIBBERELLIC ACID ON LEAF AREA AND DRY-MATTER PRODUCTION IN MAJESTIC POTATO

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(With 5 Text-figures)

When gibberellic acid (50 p.p.m. in aqueous solution) was sprayed twice or six times at weekly intervals on potato plants (var. Majestic) with a low or high nitrogen supply it did not affect rate of leaf production on the main axis, but caused earlier senescence of leaves, especially with the more frequent spraying, and inhibited leaf production and growth on laterals of the high-nitrogen plants at nodes 10 and 11 but not at other nodes. This central region of the stem appears to have a low growth potential, probably because it lies midway between two zones of active growth, viz. the basal branches and the younger leaves on the main stem. Competition between these is increased by gibberellic acid. Gibberellic acid increased leaf area even when lack of nitrogen was restricting growth but this did not produce extra dry matter. Tuber weight was increased more in high-nitrogen plants by two sprayings than by six sprayings. The net assimilation rate of low-nitrogen plants was halved by spraying but was not changed in high-nitrogen plants where the value was similar to that of low-nitrogen control plants. The high-nitrogen plants had absorbed nearly all the available nitrogen between the second and third harvests, but plants treated with gibberellic acid, nevertheless, had more total dry weight and tuber dry weight than the controls. The nitrogen content of the leaves expressed on an area basis was lower in sprayed plants and, with continued spraying, fell at the third harvest to equal that of low-nitrogen plants. Evidently, the effect of gibberellic acid depended on the interaction between the rate of application and the nitrogen supply, but further work is necessary to define the conditions that give the maximal effect on dry-matter production.

Humphries (1958) found that leaf area and dry-matter production of potato (var. Majestic) could be increased by application of gibberellic acid (GA), but there were indications that the effect did not persist until maturity. To learn more of this apparent deleterious effect the experiment has been repeated and the observations extended over a longer period of growth. As nitrogen supply is one of the most important factors determining leaf area and growth rate, the response to GA was observed on plants supplied with different amounts of nitrogen. The experiment was also designed to test the effect of spraying over a longer period to see whether renewing supplies of GA would maintain the increased growth rate.

Material and methods

The plants were raised from sets trimmed to a weight of approximately 5 g. bearing a single eye, sprouted in sand for 3 weeks, and then planted singly on 12 May in glazed pots containing 10 kg. of a fertile Woburn soil to each of which had been

added 1·9 g. K and 0·7 g. P. To one-half of the pots was also added 1·75 g. N as ammonium nitrate (cf. 1·4 g. N added in the previous experiment). Two rates of application of GA were also tested; in the first the plants received two sprayings separated by an interval of a week (treatment A), while in the other the plants received six sprayings at weekly intervals (treatment B). The GA was supplied in aqueous solution at a strength of 50 p.p.m. There were 120 plants altogether arranged in twenty blocks in trucks which were kept in the glasshouse during wet weather and put in the open air in dry weather. Compared with the previous experiment, these plants had a greater proportion of time in the glasshouse particularly in the early stages of growth. Leaf areas were estimated by a rating method on 30 May and the first spray applied. The areas were subsequently estimated as nearly as possible at weekly intervals but this operation had sometimes perforce to be spread over 3 days when leaf area was near maximum. Seven blocks were harvested after 3 weeks, seven blocks after 5 weeks and the remaining six blocks 8 weeks after the first spraying. Dry-matter and nitrogen determinations were made on the component parts.

Experimental results

Production and survival of leaves. Spraying with GA had no effect on the number of leaves formed on the main axis, but caused the formation of small compound leaves on the stem of the inflorescence, and the number of such leaves was increased by continued spraying (treatment B); thus in treatment A there was an average of 4·5 leaves per plant and in treatment B 11·0 leaves, at the end of the experiment. Unsprayed plants had no such leaves. GA did not affect the rate that leaves were produced on the main axis, but from about the fourth week caused earlier senescence of leaves, which was accelerated by spraying additional GA (Fig. 1). The rate of leaf production on the lateral branches was not greatly affected by GA except at certain nodes, but here also leaves of lateral branches survived longest on unsprayed plants. Fig. 1 shows the rate leaves were produced and how long they survived on lateral branches at selected nodes. Few leaves died at the lower nodes without GA but the death rate increased with it. More frequent spraying (treatment B) further increased the death rate of the leaves. Fig. 1 also shows that spraying diminished leaf number on the lateral branch at node 11; this produced seven leaves on the control plants but those sprayed with GA developed only three leaves, one of which in treatment A had died by the end of the experiment, whereas all had died in treatment B. The number of leaves on the branch at node 10 was similarly affected but not at nodes 9 and 12; at nodes 13–17 leaf number was increased slightly above the controls. The reason for inhibition of leaf development at nodes 10 and 11 is not certain (it is suspected that it is due to certain growth correlations), but will be considered further in connexion with leaf areas.

The total number of leaves produced on the main axis of the nitrogen-deficient plants was slightly less than with high nitrogen, and no inflorescence formed. Leaves died sooner when sprayed with GA but there was little difference between the two spray treatments.

Leaf area. Fig. 2 shows changes in area of leaves and the total area of the leaves on each lateral branch of the high-nitrogen plants. The area of each leaf on the main axis is plotted against the number of the subtending node for each weekly observation. At the end of May, when leaf area was first estimated, the 9th and 10th leaves were the largest and lateral growth was just commencing at nodes 4–8. The plants

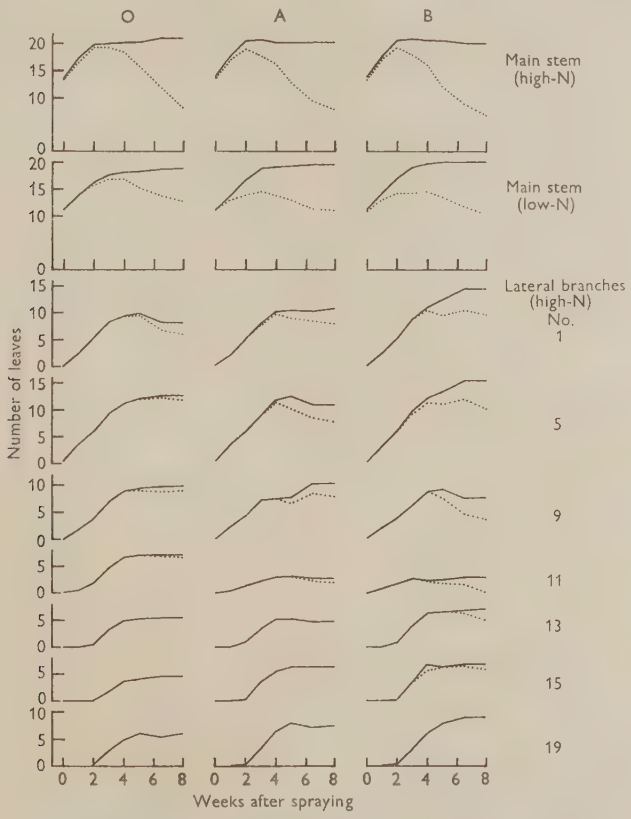

Fig. 1. Total number of leaves produced (——) and those surviving (· · · ·) on the main stem of low- and high-nitrogen plants and on selected laterals of high-nitrogen plants.

received their first application of GA at this time. A week later GA had already appreciably increased leaf area; the largest leaves, nos. 12 and 13, increased in area most. After the second spraying (5 June) the increase in leaf area was considerable. Subsequently, the plants in treatment A received no more GA, but the leaves of this treatment continued to increase in area faster than the controls. The plants of treatment B, which received spray treatment until 4 July, had only a slightly greater leaf area than plants of treatment A and this greater area was confined to

the uppermost leaves of the main axis (nodes 15 upwards) and the lateral branches. (The curves of leaf area for treatment B are not shown in Fig. 2, to avoid confusion, because they correspond closely to those of treatment A.) The leaves of the lateral branches of all treatments increased steadily in area until the end of June when the total leaf area on the largest lateral (node 4) had an area equivalent to the largest

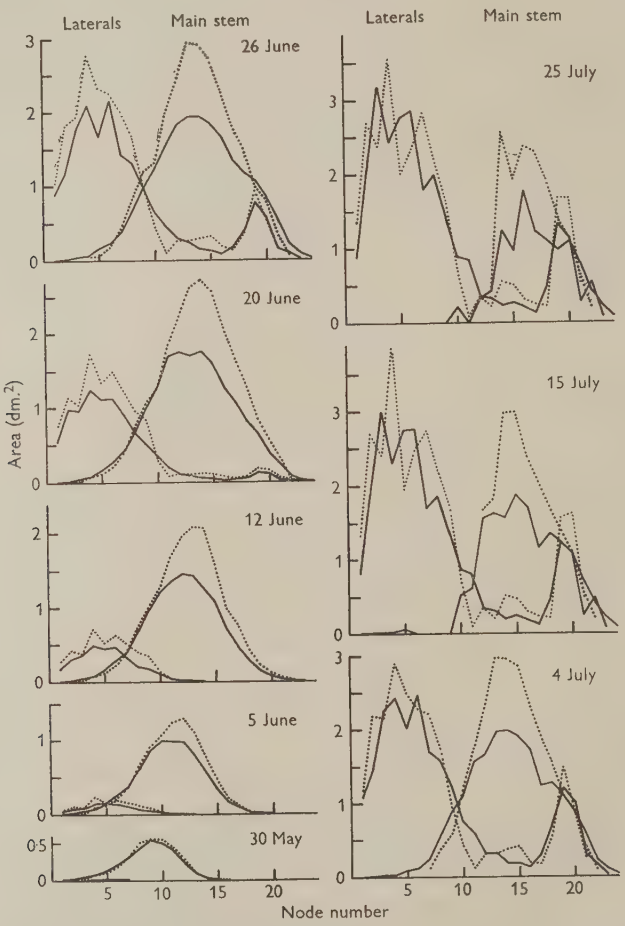

Fig. 2. Area of each main-stem leaf and total area of each lateral of the high-nitrogen plants at approximately weekly intervals. ——, control; · · · ·, treatment A.

leaf on the main axis (leaf 13 or 14). Fig. 2 shows that the leaf-area curves of the leaves of the main axis and lateral branches cross one another in the region of the 9th or 10th node and this may be considered to be the region of lowest growth potential, using this term in the sense employed by Gregory & Veale (1957). The regions

of highest growth potential were at node 4, which had maximum lateral growth, and at node 14, which had maximum leaf size on the main axis. The effect of the growth rate of leaves at nodes 10 and 11 is shown more clearly from the graph of total leaf area produced at each node at the time of maximum leaf area (26 June), i.e. the sum of the areas of the main axis leaf and of the leaves of the lateral branch subtended by that leaf. Fig. 3 clearly shows that the area at nodes 3–15 is fairly constant at about 200 cm.^{2}. This suggests that, at the time of maximum leaf area on the main axis, there is a fairly uniform and maximum demand at each of these nodes for nutrients. Plants treated with GA, on the other hand, did not show this uniformity and two maxima occur; see Fig. 3 (the average effects of the A and B treatments are shown as they were practically identical). The first maximum is at

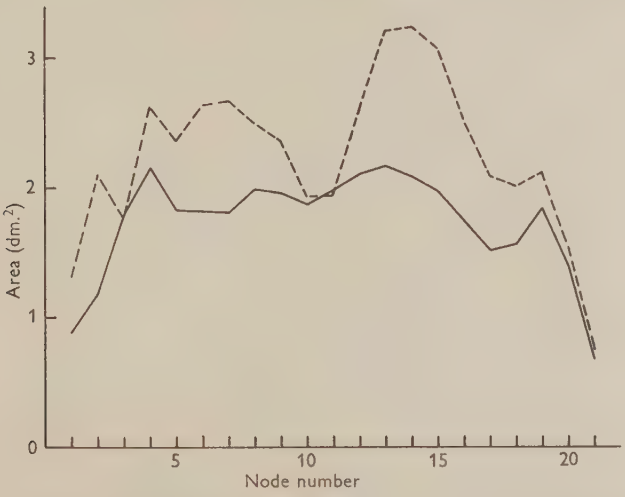

Fig. 3. Total area of main-stem and lateral-branch leaves at corresponding nodes on June 26. ——, control; – – –, mean of A and B.

nodes 6–7 and the second at nodes 13–14, and in these two regions the demand for nutrients will be increased. Spraying had no effect on total leaf area in the intermediate regions at nodes 10–11 and it is suggested that growth was restricted in this zone because of increased competition both above and below. The restriction of growth was sharply delimited in this region of the main axis.

Fig. 4 shows the changes in area of the leaves of the low-nitrogen plants which produced no lateral branches. In spite of the limitation on growth imposed by the low-nitrogen supply, sprayed plants had a larger leaf area and extra spraying produced little additional effect. Thus, GA increased leaf area even when nitrogen was deficient, but, as will be seen later, this did not produce extra dry matter.

Fig. 5 compares the effects of treatment with GA on the change with time in total leaf area of plants with high and low nitrogen supply. The maximum leaf area on

the main stem was reached on 26 June whatever the treatment and then declined, but leaf area on the lateral branches of the high-nitrogen plants increased until the end of the experiment. Later spraying (treatment B) affected leaf area of the lateral branches but not the area of the main-stem leaves. Although the actual increase in total leaf area per plant was less in the low-nitrogen plants, the relative increase by GA was similar at both nitrogen levels, 23 and 34% in treatment A and B of the low-nitrogen plants and 26 and 29% in the corresponding treatments of the high-nitrogen plants at time of maximum leaf area, which suggests that GA influences the process of cell extension in the leaf independently of the nitrogen supply. Fig. 5 also shows that, at time of maximum leaf area (26 June), the area of the main-stem

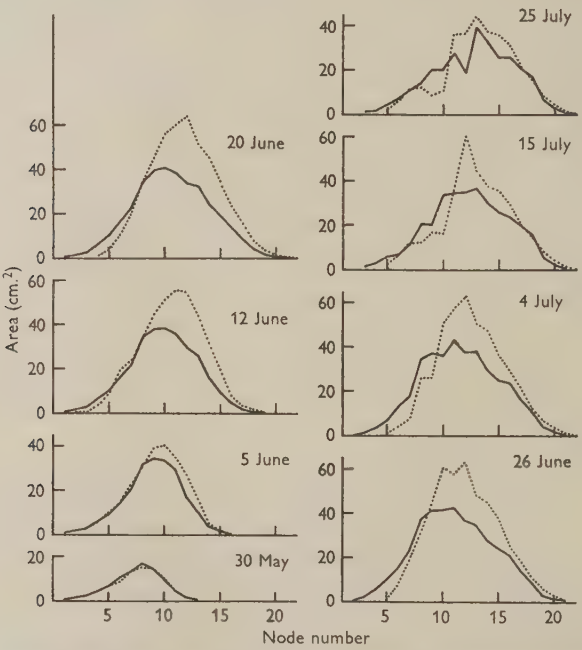

Fig. 4. Area of each main-stem leaf of the low-nitrogen plants at approximately weekly intervals. ——, control; ····, treatment A.

leaves and those of the lateral branches were approximately equal, but subsequently the leaf area on the lateral branches far exceeded that on the main stem because the former continued to increase whereas the latter decreased. At the final harvest treatment B had the larger leaf area on the lateral branches.

Leaf area and dry-matter yield at successive harvests. Leaf area and dry-matter contents of component parts at each of the three harvests are given in Table 1, which shows effects of GA on area and dry weight of leaves clearly. In the low-nitrogen plants at the first harvests GA increased leaf area by about 40% and leaf

dry matter even more. Both increases were greater than in the high-nitrogen plants (viz. 34 and 16%, respectively). At the time of this harvest two sprays had been given to treatment A and three to treatment B. The extra application produced no significant effects except a greater area of lateral leaves and there was a tendency for tuber weight to be reduced. GA had no effect on root weight in any treatment at the first two harvests, and, although differences did occur at the third harvest, their significance is doubtful because of the difficulties involved in recovering the whole of the root system.

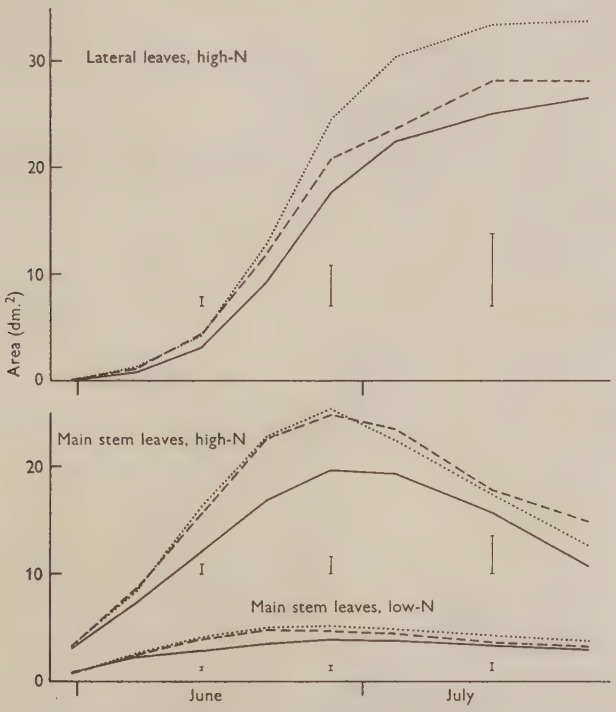

Fig. 5. Changes in total leaf area of main stem and lateral branches with time. ——, control; – – – –, treatment A; ····, treatment B. (Vertical lines indicate least significant differences.)

At the second harvest, 2 weeks later, both spray treatments increased leaf area on the main stem of the low-nitrogen plants equally. The high-nitrogen plants of treatment A maintained a greater leaf area than the controls, but in treatment B leaf area was apparently beginning to decline, probably because of more rapid senescence of leaves. These remarks also apply to the dry weight of the main-stem leaves. The lateral-stem leaves of treatment B had more area but not dry weight than the controls, whereas those of treatment A were not different from the controls

Table 1. *Leaf area and dry weight; mean per plant*

Treatment		Leaf area (dm.²)		Dry matter (g.)						
		Main-stem leaves	Lateral leaves	Total	Main-stem leaves	Lateral leaves	Main stem	Lateral stem	Tubers	Roots
				First harvest—20 June						
Low N	O	3·6	—	4·0	1·3	—	0·8	—	0·8	1·1
	A	5·1	—	4·8	2·0	—	1·0	—	0·8	1·1
	B	5·0	—	4·4	2·0	—	1·0	—	0·5	1·0
	L.S.D.	1·09		1·13	0·42		0·21		0·80	0·31
High N	O	17·3	10·4	23·1	7·6	4·0	3·2	2·8	2·3	3·0
	A	23·2	13·1	24·5	8·9	4·1	3·9	3·2	1·7	2·8
	B	23·5	15·9	25·1	9·0	4·6	4·3	3·7	0·5	3·0
	L.S.D.	2·68		1·67	0·78		0·67		2·04	0·31
				Second harvest—4 July						
Low N	O	4·0	—	7·9	1·7	—	1·0	—	4·1	1·2
	A	4·8	—	7·3	2·1	—	1·0	—	3·3	0·9
	B	4·8	—	6·6	2·1	—	1·0	—	2·5	1·0
	L.S.D.	0·77		1·51	0·47		0·38		1·94	0·46
High N	O	18·9	24·1	51·8	8·6	8·9	5·6	7·2	17·9	3·7
	A	25·0	22·9	57·3	10·4	8·1	5·6	6·7	22·5	3·9
	B	22·6	32·3	58·9	10·0	10·1	6·3	8·6	20·4	3·7
	L.S.D.	4·24		3·44	1·56		1·56		5·17	0·46
				Third harvest—25 July						
Low N	O	3·0	—	11·6	1·6	—	0·8	—	8·2	0·9
	A	3·2	—	8·6	2·1	—	0·8	—	5·0	0·7
	B	3·8	—	10·1	2·4	—	0·9	—	6·0	0·9
	L.S.D.	1·17		2·90	0·36		0·24		2·99	0·16
High N	O	10·7	28·2	116·8	8·0	11·1	5·9	8·8	79·1	3·8
	A	14·8	29·1	125·3	9·0	10·6	5·9	8·7	87·4	3·6
	B	12·7	36·8	119·0	9·2	13·3	7·2	11·2	74·2	3·9
	L.S.D.	6·00		10·35	2·16		1·85		10·57	0·16

in either respect. Tuber weight was increased in treatment A by 26% but this was not statistically significant. The total dry weight of sprayed plants in the high-nitrogen treatment was greater than that of the controls. The lateral-stem leaves attained approximately the same dry weight as the main-stem leaves by the second harvest.

At the third harvest (8 weeks after spraying began) no treatments either in the low- or high-nitrogen plants showed significant differences in leaf area on the main axis, but on lateral stems of treatment B it was still greater than the controls. The total dry matter of the high-nitrogen plants of treatment A still appreciably exceeded that of the controls, but this difference was no longer significant. This may be because variability was more at the third harvest when only six blocks were sampled and one plant was diseased, necessitating a missing-plot computation, or because dry-matter accumulation was starting to decline as in the previous experiment. The total dry matter of plants given six applications of GA was similar to that of the controls, indicating that this treatment has a deleterious effect on dry-matter production. The dry matter of the main-stem leaves was still significantly greater than controls in the low-nitrogen plants but not in the high. The lateral leaves and lateral stems of treatment B had a greater dry weight. From Table 1 it is also apparent that the maximum leaf area and leaf dry matter of the low-nitrogen plants was almost attained by the first harvest.

TABLE 2. *Net assimilation rate* (*g./dm.²/week*)

Treatment		20 June–4 July	4–25 July
Low N	O	0·52	0·35
	A	0·25	0·14
	B	0·22	0·26
High N	O	0·44	0·53
	A	0·41	0·50
	B	0·39	0·39
	L.S.D.	0·088	0·198

Net assimilation rate. The net assimilation rates (E) calculated over the two periods of the experiment are shown in Table 2. Spraying at either rate lowered the efficiency of the low-nitrogen but not of the high-nitrogen plants, which agrees with the results of the previous experiment (Humphries, 1958). There was no difference between net assimilation rates of unsprayed plants at the two nitrogen levels; although in the second interval there was an indication that nitrogen increased net assimilation rate. Thus, GA reduced the assimilatory efficiency to about one-half in low-nitrogen plants, whereas low nitrogen itself did not reduce the efficiency. Watson (1952) showed that slight nitrogen deficiency had no effect on E but severe nitrogen deficiency lowered it. The present results are in accord with the hypothesis that GA induces nitrogen deficiency, which suffices to lower E when nitrogen supply is already low.

Tuber number. GA had no effect on tuber number at the first harvest but by the second harvest treatment A (but not B) increased it in the high-nitrogen plants and

treatment B (but not A) in the low-nitrogen plants. At the third harvest only treatment B of the high-nitrogen plants increased tuber number. Why GA had smaller effects than in the previous experiment is not known. The tuber number in the present experiment varied greatly (79% of the mean of the first harvest).

Nitrogen content. The nitrogen contents of the plants at each harvest were determined after bulking samples to give three replicates. At both levels of nitrogen plants attained about 70% of their final nitrogen content by the first harvest (Table 3). The low-nitrogen plants increased slightly in nitrogen content until the final harvest but the high-nitrogen plants, which were supplied with 1750 mg. nitrogen, had absorbed about 1600 mg. by the second harvest and the amount of

TABLE 3. *Nitrogen content (mg.) per plant and per unit area of leaf*

		(*a*) Total per plant	(*b*) per dm.² of leaf: Main leaves	(*b*) per dm.² of leaf: Lateral leaves
		Low nitrogen		
1st harvest	O	79	11·6	—
	A	85	9·2	—
	B	82	9·6	—
2nd harvest	O	106	11·2	—
	A	94	8·6	—
	B	94	8·1	—
3rd harvest	O	118	11·0	—
	A	106	11·8	—
	B	124	10·3	—
		High nitrogen (1750 mg. additional N per plant)		
1st harvest	O	1140	26·4	29·5
	A	1158	21·1	22·5
	B	1169	20·4	19·8
2nd harvest	O	1639	20·3	23·2
	A	1630	17·5	21·0
	B	1608	17·1	15·8
3rd harvest	O	1602	17·1	16·0
	A	1699	14·8	13·8
	B	1648	15·2	11·0
		1957 experiment* (1400 mg. additional N per plant)		
1st harvest	O	679	32·2	
	A	701	23·1	
2nd harvest	O	1068	21·5	
	A	1114	17·3	

* Humphries, E.C. (1958).

nitrogen per plant did not increase at the third harvest. The control and sprayed plants therefore had absorbed all the available nitrogen between the second and third harvests but, in spite of this, plants treated with GA had a higher total dry weight and a higher tuber dry weight than the controls. In the low-nitrogen plants spraying had no effect on dry weight or nitrogen content, and there is no doubt that nitrogen was severely limiting growth. Nevertheless, GA had a big effect on leaf area.

The nitrogen analyses of the 1957 experiment (Table 3) are similar to those of the present experiment. In the previous experiment 1400 mg. of nitrogen was supplied, and the low recovery suggests that nitrogen was lost by leaching because the plants were grown in the open and subjected to several heavy rain-storms. This limitation of the nitrogen supply may have caused the fall-off in growth found particularly in the sprayed plants; it was thought previously to be due to a deleterious effect of GA.

The nitrogen content of the leaves, expressed on an area basis (Table 3), was reduced by spraying. There was little change with time in nitrogen per unit area of leaf when nitrogen was limiting, but there was a steady decline in nitrogen per unit area in the high-nitrogen plants; the lateral leaves of treatment B at the third harvest had a nitrogen content of the same order as low-nitrogen plants. It is clear, therefore, that leaves increased in area by GA fail to maintain their nitrogen content and may finally become deficient, and if the deficiency is sufficiently acute it may reduce carbohydrate manufacture. It is not known whether the fall in leaf nitrogen is due to limitation of absorption through the root or restriction of supply.

Discussion

This experiment again demonstrates that the dry weight and yield of potato plants can be increased by increasing leaf area with GA. Two sprayings at an interval of 1 week gave a bigger increase than did six sprays at weekly intervals. Only two rates of application were tested in this experiment, so the optimal conditions for application of the sprays may not have been achieved. There are also indications that the relation between nitrogen supply and the amount of GA applied is of great importance. Earlier death of leaves on the sprayed plants is probably an indication of nitrogen shortage. GA might have increased dry weight even more had the nitrogen supply been more liberal, but this does not necessarily follow, because GA had little effect on root size and therefore it is not known whether the plant would be able to respond to the growth stimulation of GA by increased absorption of nitrogen even if available. It is hoped to test this in future experiments.

The branching behaviour of the potato plants used in these experiments follows a very definite pattern. The basal branches are large, gradually diminishing in the central region of the stem (Fig. 2) and the anterior branches increase in size, but never attain the size of the basal branches. This inhibition of lateral growth in the central region of the stem is of interest in connexion with the problem of apical dominance. Whatever the inhibition, spraying with GA evidently aggravates the situation. It has been suggested (p. 181) that this is an example of correlative inhibition occasioned by a limited supply of nutritive material, the flow of which is directed to the most active region of growth which, in this instance, is first, the developing basal branches, and secondly, the anterior main-stem leaves. It appears that these two regions are in competition with one another and with the region between where growth inhibition occurs. Thus, there is a gradient of growth potential in the stem of potato similar to that of flax, described by Gregory &

Veale (1957), who adduced evidence strongly supporting the nutritive hypothesis of correlative inhibition. At the same time they point out that auxin also plays a part in controlling the vascular traces to the axillary bud; high auxin content may impede the formation of provascular strands to a leaf and lead to nutrient starvation.

We are grateful to Heather Pellant and Christine Glenister for technical assistance.

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REVIEWS

Vistas in Botany. Edited by W. B. Turrill. Pp. 547. London: Pergamon Press Ltd. 1959. £5.

The bicentenary of the foundation of the Royal Botanic Gardens, Kew, was celebrated in the summer of 1959 and *Vistas in Botany* is a volume commemorative of the occasion. A spell of 200 years is fairly long, by many standards, but it is not the antiquity of Kew which is remarkable, for it is a comparative newcomer in the botanical field. More than 200 years earlier (in 1545) the University of Padua had established a botanic garden, and many other universities were not far behind. The purpose of these gardens was primarily for the supply and study of drugs, so that botany served merely as a handmaid to medicine. It is interesting to recall, in this connexion, that the Apothecaries' Company of London set up, at much the same time and for the same purpose, a botanic garden which, under different management, still carries on at Chelsea the good work of botanical research and teaching. The special importance of Kew lies in the scale of its development and in the notable part played by it in furthering a wide range of botanical interests, both academic and applied.

It was in 1759 that a botanical garden was added to the royal pleasure garden at Kew (by the mother of George III); later, the garden at Kew was combined with a neighbouring royal garden at Richmond—whence the plural 'Kew Gardens'—and thereby the present area substantially reached. But it was only after 1841, when the gardens had passed to state ownership and came under the direction of those two eminent botanists, Sir William Hooker and his son Sir Joseph, that the main development of Kew as a centre for the study of plants on a world-wide basis really began. The second half of the nineteenth century was a period of intense geographical exploration; plant collectors searched far and wide and, as a result, a mass of material was sent back to Kew where it was described, classified and, when suitable, cultivated. Hence the stream of floras, monographs, plant lists, scientific reports, etc., which have issued, and continue to issue, from Kew Gardens. Hence also the imposing collection of plants in cultivation there and, what is still more important to the student of taxonomic botany, the incomparably rich herbarium. The latter, with its attendant library, is the Mecca of all students of botanical systematics.

For historical reasons, therefore, the contributions of Kew to botanical learning have been mainly in taxonomy and associated topics—such as plant geography, evolution of plants, palaeobotany, morphology. Some more recent fields of research—cytology and genetics, bacteriology, virology, biochemistry, and some important aspects of mycology and physiology —have received little attention at Kew, though there are good grounds for thinking that a considerable widening of scope is envisaged for the near future. Nevertheless, it was decided, and rightly so, that the aim of the commemorative book should be to give, as nearly as might be, a 'general picture of botany in its modern aspects'—hence its seventeen essays, each of them self-contained and giving a 'vista' of some major field of botanical study. The treatment in the majority of cases is historical, starting at the small beginnings and following up successive developments and offshoots until the present position is reached, and finally indicating lines on which further advances may be expected. In subjects which are of more recent development and which have not so far come within the Kew ambit, treatment is less extensive and has been largely concentrated on particular facets of current research. There is a bibliography to each essay (except that on plant physiology) and, as might be expected, the bibliographies are fullest for the traditional Kew subjects; for example, there are 196 references in 'Taxonomy; General Principles and Angiosperms', 234 in 'Plant Geography' and 353 in 'Pteridophyta'. These figures will convey some idea of the thoroughness of treatment adopted and, altogether, the reader cannot fail to be impressed by the mass of information that has been collected and collated in this book. It is not possible, of course, for a reviewer who is not a 'general botanist' (if such a person exists nowadays) to judge

critically the views advanced in all the contributions, but some general comments, relating mainly to the mode of presentation, may be put forward.

A pleasing feature of the text is that the authors nearly always take the trouble to define clearly any special technical terms that they use, with the result that the reader has no great difficulty in following the argument even when the subject is unfamiliar to him. This being so, the book can be confidently recommended to all botanists, research workers or teachers at all levels, who wish to have a picture of the whole subject as it stands today. Some of the essays are more readable than others, either because the arrangement is clearer or because the language is simpler. In some, the treatment is in the matter-of-fact manner of the text-book, in others the individuality of the author comes out strongly. Of the latter type is 'Bacteria', which queries much of the standard doctrine, both in the classification of bacteria and in everything else that belongs to them. But the highlight of the book, to one non-taxonomic reader at least, was the discussion of general principles of taxonomy (with special reference to flowering plants), a discussion which combines impressive knowledge with acute criticism and a highly attractive style of writing. Conversely, it may be that the taxonomically experienced reader may find his greatest interest in the non-taxonomic chapters; the highlight for him may be, for example, some remarkable electronmicrographs in the account of the viruses. If this kind of broadening effect on the reader is at all general, then one of the main objects of the book will have been achieved.

To sum up, *Vistas in Botany* is a notable book, fully worthy of the occasion of its production. Much of the credit for it must go to Dr Turrill who, in addition to acting as contributor and general editor, appears to have been largely responsible for its planning.

W. BROWN

Practical Botany for the Tropics. By W. O. Howarth and L. G. G. Warne. Pp. 238. London: University of London Press Ltd. 1959. 15*s*.

The higher levels of education provided by advanced schools, colleges and universities are becoming available to ever-increasing numbers of students in underdeveloped countries of the tropics and elsewhere, and future progress in many such areas depends largely on effective development of science, technology and agriculture. This expansion of higher education has resulted in a growing demand for text-books written specifically for use in the tropics and subtropics; this is particularly true in biology, and it is felt that the book under review will undoubtedly fill a real need in such areas.

Writers of short laboratory manuals are inevitably faced with the difficult problem of selecting the small amount of material which space permits to be included from the large amount which has to be omitted. In the present case most aspects of botany are covered in the twenty-eight rather short chapters, the last five of which (some 50 pages) are devoted to Algae, Fungi, Bryophyta, Pteridophyta and Gymnosperms, respectively. Opinions will be divided as to whether more space could profitably have been allotted to some or all of these five groups; thus neither Lichens, Discomycetes (e.g. *Peziza*) nor the Smut Fungi (Ustilaginales) appear to be mentioned. There are inevitably a few inaccuracies, e.g. *Erisiphe* and *Erisyphe* for *Erysiphe*, and the description of *Cystopus*, which typically causes small discrete creamy-white blisters and pustules, as causing a 'white, powdery, superficial covering' (p. 173)—symptoms characteristic of *Erysiphe* and other powdery mildews rather than of *Cystopus*. The twelve plates are excellent, but the absence of illustrations in the last five chapters, although no doubt intentional, may not be appreciated by some students.

Despite these minor blemishes this book should prove very useful to those studying botany in tropical areas, the more so since many such students often encounter greater difficulty with practical work than with the theoretical side of the subject. It would be almost impossible to compile a reasonably comprehensive manual of practical botany in which all the plants mentioned could be found in both wet and dry tropics, but in the manual under review an attempt has obviously been made to go as far as possible in this direction.

To the best of my knowledge this is the first published manual of practical botany written specifically for students working in the tropics, and as such will be welcomed and extensively used by students and teachers in these areas.

S. A. J. TARR

Perspectives in Virology. Edited by M. POLLARD. Pp. xix + 312. New York: John Wiley. London: Chapman and Hall. 1959. 56*s*.

This excellently produced book, profusely illustrated with many half-tone blocks and two colour plates, records a symposium held at Rutgers University in 1958 in memory of Dr F. R. Beaudette, an eminent poultry pathologist, whose special interest was virus diseases and who died of cancer. It is a fitting memorial; the subjects chosen to be discussed are most appropriate to the motive of the symposium and the speakers all have something interesting to say and mostly say it clearly. Ten of the twelve chapters deal with virus diseases of animals or man or with some aspect of cancer research; between them they not only make a valuable summary of current knowledge, but they also vividly indicate the wide ramifications of virus research and the variety of techniques now used to study viruses. It is a compliment to the organizers and editor to say that even the discussions at the ends of some of the chapters are worth reading, a rare thing in reports of meetings. Of the other two chapters, one reviews recent work indicating that the nucleic acid is the infective part of tobacco-mosaic virus, and the second describes genetic interactions between bacteriophage and bacteria. In addition, there is a Foreword by Waksman, 'Fred Beaudette as Friend and Scientist', and an Epilogue by Dubos, 'Tulipomania and the Benevolent Virus'. After the evidence for the malevolence of most viruses, so well presented in the preceding chapters, it is particularly refreshing to have the benevolence of at least one so charmingly argued. It is possible to agree with Dubos that 'size is not a very significant criterion of success' and that 'the most interesting and most creative individuals are not necessarily the healthiest members of the species'; but, even at the risk of being less interesting and less creative than they might become if infected with the appropriate virus, most people will be well advised and well content to avoid the troubles that provide the subject matter for the body of this admirable book.

F. C. BAWDEN

Progress in Biochemistry since 1949. By F. HAUROWITZ. Pp. 357. Basel: S. Karger; New York: Interscience. 1959. 60*s*. ($8.50.)

This report attempts the formidable, if not impossible, task of reviewing concisely the progress which has been made during the last decade in all branches of biochemistry. The author strikes a reasonable balance between the physical and biological aspects of the subject and invites respect for his breadth of interest. This book might have been valuable if it contained either a balanced judgement or a novel point of view. Unfortunately it possesses neither of these virtues, lacks unity and contains numerous errors.

The first chapter, Thermodynamics in Biochemistry, will disappoint biologists seeking an easy understanding of one of the most misunderstood branches of the subject. The author rightly abandons the term 'high-energy phosphate bond', which has caused much confusion in the past, but he betrays a fundamental lack of understanding by implying that the terms 'highly reactive' and 'high-energy' are synonymous. This lacuna in the author's knowledge is further exposed when he quite erroneously states that the energy released on hydrolysis of labile phosphate compounds is a measure of the tendency of the molecule to react with water. There are many other ambiguities and errors in this book, including a misuse of the term 'equilibrium' at the foot of page 32.

The reviewer was left wondering to whom this volume might appeal. It is of little value to the student, whose critical faculties it might serve to blunt rather than sharpen. It contains insufficient detail to be of value to research workers in their own specialities, and insufficient wisdom or judgement to give a balanced overall picture of the present status of biochemistry. It is doubtful whether this report will be found any more useful than its predecessor, which covered the penultimate decade. It is a matter of much regret that Professor Haurowitz should have devoted so much of his undoubted talent to producing so little of real or lasting value.

B. R. RABIN

Biochemistry of Antibiotics (Edited by K. H. Spitzy and R. Brunner), and *Biochemistry of Morphogenesis* (Edited by W. J. Nickerson), (5th and 6th Symposia held during the 4th International Congress of Biochemistry, Vienna, 1958.). Pp. 239 and 272. London: Pergamon Press. 1959. 68*s*. and 70*s*.

Cell, Organism and Milieu. (17th Symposium of the Society for the Study of Development and Growth, 1958.) Edited by D. Rudnick. Pp. 326. New York: Ronald Press Co. 1959. $8.

A Symposium on Molecular Biology, 1956–7. Edited by R. E. Zirkle. Pp. 348. Chicago: University Press. 1959. 56*s*. 6*d*.

Symposia, especially when the word is taken literally and the proceedings are suitably lubricated, are useful. They help scientists to meet and argue and to see new aspects of their own and other people's problems. It is not so obvious that they are all worth publishing. The more lucid and gregarious scientists go round from symposium to symposium and their remarks, although probably valuable in the context in which they were made, may well be repetitive when they all ultimately appear in print. Furthermore, unless the published record is part of a recognized series, it is likely to have a limited circulation. At first sight these two effects cancel out; if something has already appeared elsewhere, little is lost if it is not widely circulated. But time is wasted when a reader tracks down an out-of-the-way publication and finds that the paper sought in it is one with whose contents he is already familiar. It would be better if the more popular symposiasts would state simply that the material they presented was essentially the same as that presented, and in course of publication, at another symposium.

The four volumes under review show the different auspices under which symposia may be held. We have an International Congress, a specialist society that regularly holds symposia, and an *ad hoc* set of lectures without much unity and not, apparently, forming part of a series.

One of the twelve symposia arranged by the International Union of Biochemistry in 1958 dealt with antibiotics and it showed that, in spite of the immense amount of information we now have about them, there is still little basis for generalization. Partly this is because there is no chemical unity in the category; like vitamins and hormones, antibiotics are a heterogeneous group of substances that achieve a spurious unity because of the uses we put them to. They must be defined functionally and not structurally. It is generally, and plausibly, assumed that they act by inhibiting an aspect of bacterial metabolism that is absent, trivial, or different in man. Cell-wall synthesis is an obvious candidate because of the different significance of cell walls in free-living bacteria and metazoa. Several papers present evidence that this is indeed the mode of action of penicillin. Other papers discuss the clinical effects of mixtures of antibiotics and conclude, in spite of the fulsome claims in the advertisements of some pharamaceutical houses, that there is little evidence for synergism. Two papers on the use of antibiotics in food preservation assure us that this is both useful and harmless.

From the study of the mode of action of substances made by one organism on the metabolism of another in the presence of a third we turn, in the International Symposium on Morphogenesis, to consider some of the mechanisms by which an organism controls its own metabolism. There were four sections. One dealt, not altogether relevantly, with protein synthesis, two with control and regulation of differentiation, and one with morphogenesis in plants, animals and bacteria. Morphogenesis is also the main theme of the symposium arranged by the Society for the Study of Development and Growth. The amount of information in both these symposia should be adequate to humble any biochemist who thinks that a general picture of metabolic control is in the offing. Not only are the well-known effects of auxins and gibberellins on growth described, and to some extent interpreted, but also more specific effects, for example, the control of enzymes and the effects of salivary gland extracts on neural growth. The synthetic pattern of several different types of cell depends on the

culture medium in which they are grown and, when cells that are normally part of a tissue grow in isolation, on the presence or absence of neighbours. This type of work shows that cells do not all start out with a complete set of instructions built into them, as the DNA fanatics assume, but get much of their direction by post as they go along.

The fourth symposium has three main defects. It was held in 1956–7 so that, whatever its merits at the time, it is rather cold now; it seems to have no unity or plan; Molecular Biology is so often the title under which people strive to do biochemical research when they have little or no training in the subject that suspicions are aroused at the start. In part they are unjust; some of the authors have simply strayed into the wrong gallery.

Of all forms of scientific literature, the symposium has the shortest expectation of useful life. An authoritative book has permanent interest if the author was, at his date, influential; it crystallizes a contemporary attitude of mind. Research papers are often the only record of certain results and methods; they may be referred to for more than antiquarian reasons half a century after publication. But who, except from morbid curiosity, reads old review articles? It is extraordinary therefore that glazed paper and heavy bindings have been lavished on these four volumes: the four of them come to more than £12 10*s*. Each would have been perfectly satisfactory in the format of a number of the *Annals of Applied Biology* but this is a style our publishers dislike. They do these things, it seems, better in France. It might be well to try their publishers for symposia.

N. W. PIRIE

Plant Nematology. Edited by J. F. Southey. Pp. 175. London: Her Majesty's Stationery Office. 1959. 9*s*. 6*d*.

It is evident from conversation and correspondence with overseas biologists, especially those from the Middle East and Asia, that plant nematode problems are very serious in that part of the world. The situation is aggravated by the scarcity of trained nematologists outside Europe and America and any recruitment of nematologists for posts overseas is hindered by the apparent reluctance of most universities in Great Britain to consider plant-parasitic nematodes seriously.

The book will, therefore, be appreciated most by those biologists who, trained in another discipline, are faced with plant nematode problems. A description of the general biology and morphology of plant nematodes is given in the first section and is sufficient to enable the student to appreciate their morphological diversity and to classify individuals as far as order. Later sections of the book give illustrated accounts of the principal genera—*Ditylenchus*, *Meloidogyne*, *Heterodera*, *Aphelenchoides* and *Pratylenchus*, with brief reference to other plant-parasitic forms. The illustrations are numerous and most of them are good. The exceptions are some of those reproduced from the works of other authors. For example, in some drawings of nematodes (Figs. 40, 44 and 47), I suspect that regions of doubtful internal morphology are covered with a cloud of black dots in the guise of fat globules.

Two further sections deal with the distribution, life history, symptoms, control and research problems of the genus *Heterodera*, reflecting the importance of the group in Great Britain. A section on control describes the principles involved in soil fumigation, contact nematicides, hot water treatment of plants and the growing of eelworm-resistant potatoes. A final section, of particular interest to the experimental zoologist, refers to laboratory culture and nutrition studies.

The book was compiled from a series of lectures given at a training course primarily for N.A.A.S. officers and was never intended to be a complete treatise on nematology. However, the scope is wide enough to give the non-specialist a very good idea of some of the current problems in nematology as well as being a useful practical handbook. Those parts of the book dealing with the hatching process in *Heterodera* spp., the movement of plant nematodes in soil and with eelworm-resistant potatoes are somewhat outdated because of the inordinately long time of 3 years taken in publication, so a revised edition seems advisable in the near future. This is a minor point, however, and does not detract from the value of the book.

H. R. WALLACE

Plant Growth Substances. By L. J. Audus (2nd edition). Pp. 553, 34 plates. London: Leonard Hill (Books) Ltd. 1959. 65*s*.

Since the first edition of this book was published in 1953, much research on plant-growth substances has taken place along chemical, plant physiological and applied lines. All these aspects are covered in the new edition, and it is therefore not surprising that the book has grown from a modest-sized volume to quite a substantial work. The bibliography, for example, has increased from 23 pages in the first edition to 63 pages. The much improved production of the revised edition makes it an attractive and useful volume for the chemist, botanist and agriculturist alike.

The detailed list of contents, clearly labelled diagrams, good photographs and valuable appendices which marked the first edition remain and are supplemented, while the setting is improved by a more spacious layout.

The framework adopted in the first edition is little changed, but a new chapter has been introduced on the mechanism of action of the auxins. The chapter on auxins and the shedding of organs replaces that on auxins and tissue differentiation and the new edition includes a section on the use of synthetic chemicals to promote abscission. The preparation of new chemicals with growth-regulating activity has necessitated a considerable expansion in Chapter III, the Chemistry of Auxins (Natural and Synthetic), where the structural requirements for activity are reviewed in the light of recent findings; thus, for example, the activity of compounds not possessing a ring structure receives attention. The new Chapter IV, dealing with the mechanism of auxin action from the physiological viewpoint, while recognized by the author to be beyond the sphere of the layman, for whom the book was partly intended, serves to balance the chemical detail of the previous chapter.

Chapter V, Auxins as General Growth Stimulants, is not very different from the first edition, although a short section on the gibberellins has been included. In Chapter VI, on the rooting of cuttings, recent work is discussed on conditions affecting the response and the influence of other substances. Reference to the use of growth-regulating substances in conjunction with mist technique might well have been included here, in view of its increasing use in horticultural practice. Modifications in Chapter VIII include a section on growth stimulation in normal fruits, while reference to the use of growth-regulating substances in plant breeding has been expanded. It is questionable whether Chapter XI, General Toxicity and Use as Selective Weedkillers, has not been made too wide by the inclusion of selective herbicides, such as dinitro-ortho-cresol, which are not of plant growth-regulating type. Chapter XIII, Hormones and Reproduction, has been considerably lengthened, especially in relation to the role of auxin and gibberellin-like factors in flowering. A section on the recent and rapidly developing techniques of tissue and organ culture appears in Chapter XIV, Specific Factors for the Growth of New Organs.

The six appendices represent useful collections of data on response of cuttings, etc., to hormone treatment. They have undergone surprisingly little change compared with the former edition with the exception of a new item in which apple varieties are listed in relation to their response to α-naphthylacetic acid in treatment against pre-harvest drop.

The book is remarkably free from printing errors. In Appendix A, Guava (*Psidium guajava*) appears twice—as a fair rooter and as a good rooter when untreated. There is an error in the formula for indole-3-methane-sulphonic acid on page 68.

The standard of presentation is excellent throughout. Fig. 20 might, however, have been clearer if the many arrows had been omitted and Table II would have been improved by a heading stating what the figures are, although the explanation in the text is close at hand. The concentration of the chemicals used to obtain the experimental results shown in Plate 5 might have been stated. These, however, are small criticisms.

All concerned with this field of research will welcome this new edition which brings a subject with so many facets up to date.

M. S. SMITH

MÜLLER, P. (general editor): *DDT, the Insecticide Dichloro-Diphenyltrichloroethane and its Significance.* Vol. I. Ed. by PAUL MÜLLER. Pp. 299. Basle and Stuttgart: Birkhäuser A.G. 1955. 37·5 Sw. fr. DM. 37.5.

Seventeen years have passed since the insecticidal properties of DDT were disclosed to workers in this country. During this period the efficiency of DDT in controlling many insects affecting plants, animals and man received world-wide recognition. No other insecticide has aroused greater or more sustained interest and it is most appropriate that a comprehensive monograph on the development and application of DDT should be compiled under the editorship of Dr Paul Müller. Two volumes of the monograph have now appeared.

Research workers of international repute from different countries have contributed to the monograph. In the first volume, P. Müller gives an account of the discovery and history of DDT and of its spectacular use during and after the Second World War in the control of disease-transmitting insects. This is followed by a full description, by the same author, of the physical and chemical properties of DDT, of analytical methods, synthesis and industrial production, of its isomers and analogues and by an examination of constitution in relation to insecticidal action. V. B. Wigglesworth, in an authoritative account of the mode of action of DDT, describes its absorption into and action within the insect body and examines the factors influencing its effectiveness, the theories accounting for its activity and the problem of acquired resistance by the insect. E. Bernfus deals with the uses of DDT in controlling insects attacking stored grain and reports findings on the levels of contamination of processed food commodities. The protection of textiles is discussed by O. Wälchli, who describes the biology and physiology of wool pests, methods of testing for proofing and the duration of effect of DDT preparations. V. Butovitsch contributes a comprehensive article on the place of DDT in forest management. He describes the materials and methods of application used for the control of forest pests, relates practical experiences, gives the relative susceptibilities of different types of forest insect and shows that losses of non-harmful members of the forest fauna can be reduced to a minimum if suitable materials are properly applied at appropriate dosages and times.

The volumes are attractively produced, English or German is used with summaries in both languages and each contribution gives many references. The monograph worthily commemorates the many achievements of DDT.

J. T. MARTIN

MÜLLER, P. (general editor): *DDT, the Insecticide Dichloro-Diphenyltrichloroethane and its Significance.* Vol. II. Ed. by S. W. SIMMONS: *Human and Veterinary Medicine.* Pp. 570. Basle and Stuttgart: Birkhäuser A.G. 1958. 66 Sw.fr. DM. 66.

The second volume of this series contrasts in several ways with the first. It is substantially longer, running to 570 pages as compared with 300; yet there are only three chapters instead of five. Whereas the contributors to the first volume were all European, the second volume was compiled by three Americans. The two longest sections deal with the toxicity of DDT to man and the use of DDT against insects of public health importance; the third section considers the veterinary uses of the insecticide. These three subjects are fairly closely linked and the extensive treatment of them (based on 685, 668 and 203 references, respectively) reflects the very great contribution made by DDT to the control of pests of man and domestic animals. Although DDT is extensively employed for other purposes, its outstanding virtues are selective contact toxicity to arthropods combined with long residual action. These properties are especially valuable for attacking ectoparasites and disease vectors.

The review of DDT hazards by Dr Hayes is well balanced and comprehensive. It is somewhat inconclusive in places, partly because of different results by different experimenters but largely because of the ignorance which still surrounds the lethal action of DDT. Thus,

while it is highly probable that its major site of action is the nervous system, the only conclusions drawn are: '(1) Both central and peripheral effects have been observed. (2) No unifying theory is available which will explain the observed effects.' Full details are given of the levels of contamination likely to be harmful and the more alarmist suggestions of a mysterious 'X-disease', due to widespread DDT poisoning, are refuted.

Dr S. W. Simmons (who acts as editor of this volume) has written a long section on the use of DDT against insect pests affecting man. Most of these are dealt with on a geographical and historical basis; so that, for example, the use of DDT for malaria control is reviewed country by country rather than as a whole. Much of the earlier history is put on record in a proper perspective.

Dr E. F. Knipling, dealing with veterinary insect pests, begins with a short review of methods of applying DDT (dips, sprays, etc.) and then considers the various types of pest (flies, mosquitoes, ticks, etc.). This follows the same pattern as the preceding chapter and one wonders whether it might not have made an interesting contrast to arrange this section according to different types of livestock involved rather than on an entomological basis. However, the chapter is adequate and well illustrated.

Considering these books as a whole one can see greater value in some sections than others. A chapter on the toxicology or the physical characters of DDT can be of value without reference to any other insecticide. But an author reviewing any given field of pest control must feel somewhat limited in being restricted to a single insecticide. Probably it would not be possible to attempt anything on this scale with any other insecticide.

The most serious defect of the present volume is the delay in publication. The chapters appear to have been written in 1953 and a great deal has happened in the insecticide field since then. The section on insecticide-resistance, for example, is a little weak. However, DDT was the first of the new synthetic residual insecticides and a great deal of valuable work on it was published in the first decade of its wide use. Every department interested in insecticides will need copies of these books for reference, though the prices may deter individual purchasers.

J. R. BUSVINE

Statistical Methods in Biology. By N. T. J. Bailey. Pp. 200. English Universities Press. 1959. 25*s*.

Elementary Statistics, with Applications in Medicine and the Biological Sciences. By F. E. Croxton. Pp. 370. New York: Dover Publications. 1959. 16*s*.

A new 'elementary' book is always interesting, since no two teachers agree exactly on how the subject may be presented to non-specialists. It is a little difficult to decide where Dr Bailey's book fits in to the body of statistical literature, for the ideal reader would be one who had become familiar with the concepts and methods of expression of elementary statistics and did not have to halt or even to slacken pace at each appearance of a new technical term. Dr Bailey throws these terms in casually as occasion demands, and passes them off as if we had been living with them since birth; this is no doubt a deliberate attempt to divest statistics of the false terrors that it holds for many scientists, and if it succeeded would be very praiseworthy. The reviewer felt, however, that many of the biologists who might turn to this book would find themselves in a foreign country with a manual of folk-idiom rather than a phrase-book. He therefore tried this out on a very small sample: two people with some statistical background, but who do not use statistical methods very often, and two research workers of some experience but anxious to acquire statistical knowledge. The latter, according to the dust-cover, are the sort of people for whom the book is written, but they felt unable to gain very much from it: although there are no mathematical complications, the technical jargon defeated them because it was not explained in enough detail. But the people with a statistical background, and the reviewer himself, will certainly use this book as a ready work of reference and will gain in doing so.

Professor Croxton's book is a reissue of a 1953 publication with a slightly different title,

and covers what is usually accepted to be the basic elementary mathematics of statistical methods: frequency distributions, location and dispersion, normal, binomial and Poisson distributions, correlation and regression, standard significance tests. It does not go so far as experimental design, and so cannot be said to cover 'biological statistics' as a subject: indeed its title is quite accurate, for it explains the elementary methods with the aid of examples taken from the stated fields. The level of mathematics required is moderate, including a fair amount of algebra but not calculus.

G. M. CLARKE

Subcellular Particles. (5th Annual Symposium publication of the Society of General Physiologists in America.) Edited by TERU HAYASHI. Pp. viii + 213. New York: The Ronald Press Co. 1959. $6.

The understanding of biochemical activities in living systems is increasingly dependent on the knowledge which is being discovered concerning the structural properties of intact cells and their component entities. It is unavoidable, however, at the present time that to observe cell structures in their ultimate state of resolution, the cells themselves must be disrupted and subjected to highly artificial surroundings. This 'impasse' in elucidation has naturally encouraged pessimistic criticism of studies of this sort. For this reason, among many, the introductory chapter by Dr Alex B. Novikoff on 'Approaches to the *in vivo* function of subcellular particles', is especially apposite in relegating those he terms 'gloomy critics' to their proper place where they may be heeded but not allowed to negate the value of attempts to observe subcellular organization.

The chapter by Dr George Palade on 'Functional changes in the structure of cell components', is an outstanding example of how correlation between the structural entities and the biochemical properties of the fractions in which they occur under specified physiological conditions, has led to the likely demonstration of the biochemical identity of recognizable subcellular particles. The conclusions reached from use of the most advanced techniques of electron microscopy virtually confirm the original idea of Heidenhain in 1875, a romantic, yet instructive and salutary result! Palade's observations lead to further exciting indications concerning the mechanisms whereby disruptive cellular activities are controlled in the physiologically organized cell.

Dr C. de Duve reviews the evidence for the existence of the lysosomes as subcellular entities distinct from mitochondria and possessing specific biochemical functions; especially those involved in acid hydrolysis. The methods described by which this evidence is obtained include comparative studies on enzymic activities shown by particles separated by differential centrifugation in deuterium (D_2O) sucrose solutions, and electron-microscope observations on their morphological characteristics.

Dr David Green describes the resolution of the mitochondrion into fragments which are morphologically distinguishable, and which can be associated with specific and different biochemical activities. The capacity for oxidative phosphorylation is related to the preservation of the double membrane structure observed in the cristae, whilst the electron transport particles are vesicular single membrane structures which cannot promote phosphorylation. Especial attention is also given to the role of lipids in the function and structural arrangement of mitochondrial proteins, and to the new manifestation of enzyme activities following mitochondrial injury.

Dr E. L. Kuff and Dr A. J. Dalton describe recent work on the Golgi membranes obtained from rat epididymis. Enzymic properties are conspicuous by their absence and so far only acid phosphatase and a high lipid content have been associated with isolated Golgi membranes. The conclusion is considered that these structures, which may comprise organized layers of membranes bounding flattened vesicles, function in secretory or absorptive capacities.

Two chapters are of special concern to plant physiologists. One by Dr Siegel describes a novel approach to the study of surface orientation factors affecting polymerization as observed in lignin synthesis in model systems. The other, by Dr Martin Kamen and Dr J. W. Newton, presents some views on photosynthetic phosphorylation. It is unfortunate that the

space devoted to this chapter is so small that many points lack the necessary detail for their significance to be understood. The statement that photosynthetic phosphorylation is an anaerobic process which is inhibited by oxygen should be qualified by reference to the system concerned, since in higher plants the process is oxygen-independent. The reviewer obtained the impression also, in spite of some citations, that more recognition might be given to the major contributions of Arnon and his collaborators in the field.

The problems of enzyme reactions at surfaces are dealt with in detail as a physico-chemical problem by Drs A. D. McLaren and K. L. Babcock. Important differences in hydrogen ion concentration between bulk phase and surface phase systems may determine a number of cell-enzyme activities and the surface or bulk phase state of enzymes may correspondingly alter their charge and activity, but not apparently the reactions they perform.

A different approach to the study of nuclear activity is presented by Drs J. Herbert Taylor and P. S. Woods, who describe *in situ* studies on polynucleotide synthesis in nuclei and chromosomes. It appears that DNA synthesis may take place over a period of time in any given chromosome. Autoradiography with tritium-labelled thymidine reveals unevenly labelled single chromosomes. The conclusion is also reached from a most elegant experiment with *Drosophila*, that the nucleolus, which is practically devoid of DNA, is a site for rapid synthesis of RNA independently of the activity of the chromosomes or nuclear cytoplasm. Some evidence is also presented for at least two general types of RNA which are respectively synthesized rapidly and slowly.

Drs Stephenson, Heckt, Littlefield, Loftfield and Zamecnik describe the recent development in the study of intermediate reactions in protein synthesis with special reference to the function of soluble RNA in binding activated adenyl-amino acids. The proposed scheme ingeniously accounts for the requirement for both ATP and other specific nucleoside triphosphates and in all reactions a reversible pyrophosphorylase action is postulated. The absence of amino-acid competition indicates the existence of specific polyribonucleotide units for each amino acid. These in turn are considered to transfer amino acids to bound RNA of the microsomes and the role of guanosine triphosphate is considered a possibility at this stage. The formulations in this chapter are occasionally lax since adenyl-amino acids are apt to lose this grouping in the text. One might also sigh for a centrifuge which would separate microsomal from soluble fractions at 15,000 *g*.

The book, for which the editor, Dr Teru Hayashi, is to be congratulated, is of outstanding interest and ranks as a major contribution both as regards the presentation of current knowledge and the formulation of highly stimulating ideas.

E. J. HEWITT

Comparative morphology of Vascular Plants. By ADRIANCE S. FOSTER and ERNEST M. GIFFORD, Jr., 555 pp.; 213 illus. San Francisco, California, U.S.A.: W. H. Freeman and Company; London: Bailey Bros. and Swinfen. 1959. $9. (76*s*. 6*d*.)

In this volume, two distinguished American botanists have attempted to deal with the comparative morphology of vascular plants, in a manner suitable for students at the senior or graduate level; the course of study to comprise about thirty lectures and 90 hr. of laboratory work. Anyone who is at all familiar with the scope of the subject will realize what a difficult task the authors have undertaken: the subject is of long standing; the materials for study, with the exception of the fossils, are more than abundant in some of the major groups; and the leading concepts and theories have been much discussed during many decades. Thus, at the outset, there are evidently important questions as to how much factual evidence should be set out, how it should be introduced, and to what extent both particular and more general theories and trends should be discussed. It is certain that the authors have appreciated the magnitude of their task and that they realize the difficulty of satisfying the many different kinds of reader who may turn to this book. It may, indeed, be doubted if justice can be done to the subject in what is a relatively short text; but, in these days, students' texts must after all be kept within reasonable limits.

Within the unavoidable limitations of space, the authors have produced a lucid, interesting and informative text in which the many aspects of plant morphology have been described and discussed. It may be said that plant morphology is once again on the move; and although some readers might consider a more detailed and comprehensive treatment of certain facets of the subject desirable, this book will, nevertheless, give the student a useful and probably adequate introduction to an extensive and important field of study. In the hope of achieving this, the authors have devoted the first six chapters to the general principles, scope and objectives of plant morphology. These chapters, for example, include some discussion of the concepts of homology and homoplasy, the basis of comparative morphological interpretation, factual and theoretical considerations relating to the main vegetative and reproductive organs, and the value of the evidence that can be obtained from the fossil record and from studies of ontogeny.

The authors have called attention to the important relationships between physiology and morphology in terms of organography as defined by Goebel in the earlier edition of his great work, i.e. the relation of form and function. They have also briefly indicated the aims of contemporary work on morphogenesis and the need to investigate the several factors—genetical, biophysical and biochemical—which determine both general and particular patterns of development. Considerably greater emphasis might well have been placed on the physiology of development; for that, indeed, is what many morphologists and physiologists regard as a central theme in contemporary botany; and such a treatment would almost certainly have done a service to plant morphology. This causal approach has an important bearing on the treatment of the subject matter of later chapters in which the vegetative organs of the sporophyte are being considered. Topics such as the origin and nature of microphylls, macrophylls and branching could have been presented in a more stimulating and realistic manner had causal as well as comparative morphological interpretations been advanced. In short, nothing is so good as a thorough-going morphogenetic approach to the problems of morphology in the evaluation of the theories of an earlier period, many of which now seem to be rather outmoded. A somewhat similar criticism might be urged regarding the treatment of comparative anatomy. Thus, whereas stelar theory has been faithfully dealt with in its classical form, no mention is made of the probable causal factors, for example, those determining size-structure correlations as they may apply to enlarging vascular systems. In the sixth of the general introductory chapters, on embryogeny, the authors have succeeded in introducing a vast field of information and in blending the phylogenetic and causal approaches.

In the remaining chapters the major systematic groups of the vascular plants are surveyed, beginning with Psilopsida and continuing on to the general morphology and evolution of the angiosperms. The difficulties which must have beset the authors in attempting to cover so wide a field are well seen in the last two chapters; for within some 100 pages they have had to compress representative information and points of view on the vast and varied group of the angiosperms, the predominant contemporary plants. This is a useful though much abbreviated summary, due attention being paid to a selection of the interesting features that emerge from morphological and anatomical investigations. It is, of course, evident that, for an adequate understanding of the flower in all its morphological diversity, and of the processes of fertilization and embryogenesis, other, more extended, works will also have to be consulted by senior students. However, the main points are there and clearly set out, although some readers may perhaps consider that this part of the book is rather less full than some of the larger elementary texts. Notwithstanding the criticisms advanced in this review, it should be said, with appreciative emphasis, that the authors have faithfully introduced their readers to the comparative morphology of vascular plants and to many of the major conceptions that have been formulated during the past century.

The book is abundantly illustrated by line drawings and photographs. The latter are excellent and often delightful, but some of the line drawings, though didactically sound, are rather unattractive.

C. W. WARDLAW

Breeding Field Crops. By J. M. POEHLMAN. Pp. 427. New York: Henry Holt and Co. 1959. $8.50.

This book gives ready access to information on practical aspects of breeding wheat, oats, barley, rice, flax, tobacco, soya beans, maize, sorghum, cotton, sugar beet and forage crops. Each crop is treated under the general headings: origin, cytogenetics, varieties, methods of breeding and objectives. Genetical principles in relation to plant breeding are presented in two short chapters, and the presentation is so condensed that it is doubtful whether the student will find these useful, except perhaps as an *aide mémoire*. A bibliography, drawn largely from North American publications, is presented separately for each chapter. The text is well written and copiously illustrated with over 250 photographs and 100 line drawings. Although the illustrated-weekly type of presentation is not to my liking, the volume is excellently produced, and is remarkably free from editorial blemishes. The work is a valuable addition to literature on crop plants, but it is to be hoped that students in British universities will not be asked to carry this kind of memory load in their training. WATKIN WILLIAMS

Rice. By J. H. GRIST (3rd edition). Pp. 466. London: Longmans, Green and Co. 1959. 48*s*.

In this new edition of a book first published in 1953, Part I deals with the origin and history of rice, its morphology and anatomy, and response to soils, climate and water supply. There are new chapters on genetics, breeding and selection of varieties. Methods of classification are described, first into the main groups: *indica*, *japonica*, *brevindica* and *brevis*; and the justification for this and other methods of classification is discussed. The genetical treatment is fairly recent and follows that of Ramiah and Rao in the Indian Council of Agricultural Research Scientific Monograph, *Rice Breeding and Genetics*.

Part II concerns the production of the crop and reviews methods of cultivation on both wet and dry lands, mechanization, nutrient requirements, and control of pests and diseases, with a new chapter on weeds. Among the points noted are the astonishing quantities of insecticides and fungicides now used in Japan and China and the increasing use of fertilizers. While it is true, as the author says, that more knowledge is required of the analysis of paddy soils and their nutritional requirements it is also a fact that such knowledge is now being rapidly accumulated.

Part III covers the period from harvesting to consumption. It deals with the storage of paddy and rice, milling processes, and nutritional values, and has a chapter on production, yields and consumption and one on economic conditions. An appendix on fish in paddy fields has been added.

The possibility of increasing the yield of paddy has been the subject of much speculation and of considerable expenditure by an international organization. Between the five immediate pre-war years and the years 1950–1 and 1955–6 increases have been great in some countries where yields were already high, for example, Spain, Australia and Japan, but negligible or non-existent in low-yielding countries such as Indonesia, Burma, Thailand and India. The author believes that 'the potential crop for the existing world area under paddy might be double that at present obtained', but progress in this direction seems to be painfully slow.

The third edition of *Rice* provides a well-written compendium that is remarkably comprehensive and balanced. A few of the spelling errors of the earlier editions remain uncorrected—'polyphiletic' for 'polyphyletic' and 'complimentary genes' for 'complementary genes'. In a book of such length it would have been advantageous to provide summaries for all, instead of some of the chapters. However, the index is good and the subheadings make it easy to find information on almost any aspect of rice in which the reader is interested. The book will certainly provide a major reference work for many years to come.

G. WATTS PADWICK

Principles of General Physiology. Vol. 1: *The Physico-Chemical Background.* By L. E. Bayliss. Pp. 520. London: Longmans, Green and Co. Ltd. 1959. 55*s.*

The last edition of Sir William Bayliss's classic book of the same title appeared 35 years ago. With so much new material to consider, Dr Bayliss has found it necessary, in revising his father's work, to split it into two volumes. The first volume deals with the physico-chemical background to physiology; and the second will describe its applications to the subject.

The stamp of the old book is still evident in some of the chapter titles and subdivisions, but the present volume is virtually a new work, in approach as well as in content. Gone are the 'frills' which made the old work such delightful if inconsequential reading. This volume is essentially a text-book of physical chemistry for biologists. Although the author is careful to point out the biological applications of the principles he enumerates, and to deal in outline with those physical and chemical techniques which are of most value to the biologist, his approach to physiology is not really biological but physico-chemical. For example, the theory of the Donnan equilibrium is explained, and its applications to nerve and muscle theory; but the question whether a simple Donnan equilibrium can, in fact, exist in living tissues is not discussed. Dr Bayliss may attempt to redress this balance in his second volume, but that will not alter this limitation on his first volume, which may be used separately.

The book is intended primarily for physiologists and experimental biologists, to whom it should be of value. The author also hopes that it may be useful to students of medicine and biology, but this is unlikely. Most students of medicine and biology have very weak backgrounds in physics and mathematics, especially in the latter. But this book is wholly mathematical in approach, and its reader requires an understanding of certain branches of advanced level mathematics to cope with it. In some chapters, notably those on The Colloidal State, Catalysis, Oxidation and Reduction, and Light and Other Radiations, it is possible to pass over the mathematics, and still to learn something from the text. But in the early and possibly more fundamental chapters on Energetics, Chemical Reaction, and Solutions, and to a great extent in the remaining chapters, this is not possible. Dr Bayliss is entitled to aim at any audience he pleases, but I feel it is unfortunate that he did not adopt a more graded approach with less mathematics in the initial stages of his arguments, which would have increased his field of potential readers.

Nevertheless, Dr Bayliss, given his limited circle of readers, caters well for them, with a clear and logical text backed by a good bibliography. The only two real blemishes are the rather sparse index and a tendency to refer back to equations previously given by means of number references without page references. The qualifications expressed above should not apply to the second volume, and I look forward to its appearance.

D. W. WOOD

Evolution of Nervous Control from Primitive Organisms to Man. Edited by Allan D. Bass. Pp. 231. Washington: American Association for the Advancement of Science. London: Bailey Bros. and Swinfen. 1959. 52*s.*

After reading the title one must ask 'nervous control...of what?' From the Preface an answer emerges, namely, the whole organism, and since the nervous system exists solely for the purpose of controlling the organism in relation to its environment this covers the evolution of all its functions—a vast topic. No more fascinating subject awaits the attention of comparative neurophysiologists, but awaits is surely the operative word, for as yet the information on the subject is negligible. The book contains nine papers prepared for a symposium held in 1956, which might well have focused attention on the need for research on comparative aspects of nervous functioning and stimulated investigations on evolution in nervous systems. But from the assembled papers it can only be concluded that the participants were not well chosen. With perhaps two exceptions they have not understood the import of the title of the symposium and have presented run-of-the-mill review accounts of their latest work in a broad variety of fields, from embryonic induction

to psycho-analysis, without attempting to meet the requirements of the subject. There is a very fine paper by Grundfest on Evolution of Conduction and useful reviews are to be found by Koelle on Neurohumoral Agents as a Mechanism of Nervous Integration and by Page on Chemistry of the Brain. General readers will find the volume interesting and stimulating in places, but they are likely to be little enlightened regarding the nature of nervous control mechanisms and less still as to their evolution.

GRAHAM HOYLE

The Principles of Humane Experimental Technique. By W. M. S. Russell and R. L. Burch. 238 pp. London: Methuen and Co. Ltd. 1959. 30*s*.

This book is concerned with yet further improvement in the welfare of experimental animals. The starting point is a careful analysis of what animals are used, where, and for what purposes, based on the material collected in 1952 by the Laboratory Animals Bureau. The analysis brings out the astonishing preponderance of the use of mice, and highlights the numerical importance of certain procedures, such as insulin assay (by induction of convulsions in mice) and toxicity testing in general, about which one cannot help feeling slightly uncomfortable. Suggestions are made for alternative procedures, with particular emphasis on the choice of experimental material.

The purpose of an assay is to provide a quantitative prediction of the effect a material might have if used for therapeutic purposes. The test-object in the assay acts as a model of the intended final recipient. In choosing a test-object one should therefore ask: 'in how many, and in what specific, characters should the test-object resemble the intended recipient?' There is a well-documented discussion of the relative claims on the one hand of 'overall similarity of phenotype' (which would lead logically to the use of man as the principal experimental animal for assays) and on the other hand of 'specific sensitivity' (leading to the increased use of insentient material such as tissue cultures or micro-organisms). Important evidence is also set out as to the gain in experimental precision, in the form of reduced variance, which can follow careful attention to the husbandry side of animal management. The use of trained animals is an important particular case.

Finally, there are valuable suggestions on the application of recent findings in the ethology of birds and fishes to the problem of assaying drugs intended to reduce fear or anxiety. One can study effects on an animal's instinctive hesitations instead of effects on the rate of development of fear during deliberate punishment.

The opening sections are the weakest part of the book, written in a distressingly 'undergraduate' style. To avoid being put off altogether the reader would be well advised to skip the first thirty pages or so. The later sections are clear, interesting, and important. It is to be hoped that many people will read them, particularly those who control laboratories where large numbers of animals are used.

T. D. M. ROBERTS

Photoperiodism and Related Phenomena in Plants and Animals. Editor Robert B. Withrow. Pp. 922. Washington: American Association for the Advancement of Science. London: Bailey Bros. and Swinfen. 1959. £6 13*s*.

These Proceedings of the 1957 Gatlinburg Conference on Photoperiodism comprise more than fifty separate contributions. They include articles reviewing whole sectors of the field, but the majority are concerned with the presentation of the then (1957) recent experimental results. Some of the review papers are outstanding, e.g. the kinetic analysis of photoperiodism by the late Dr Robert Withrow, the editor of the volume. A wealth of detail is contained in the nearly 1000 pages, and the aspects discussed by different contributors range over such diverse topics as the photochemical principles, the factors controlling seed germination and flowering in plants, the breeding cycles of birds and mammals, the effects of plant and animal hormones, and the relations between environment and internal rhythms in plants, higher animals and man.

Inevitably, there is a good deal of overlap among related papers. Since it is often instructive to see the same facts through the eyes of different workers, this is not necessarily a disadvantage, but it can be bewildering to those not familiar with the subject. As Professor Bonner states in the article introducing the section of photoperiodic control of reproduction in plants, it is probably impossible now for one individual to remember simultaneously all the relevant facts in this field, and to the research worker this record of the Conference is clearly of very great value. On the other hand, the rate of advance of research in as active a field as this is likely to make many of the contributions out of date very shortly, largely by the more recent efforts of the contributors themselves. The long-term usefulness of publishing the proceedings of conferences of this nature is therefore somewhat doubtful, especially if the publication cost is as high as in this instance.

W. W. SCHWABE

The Scientific Principles of Crop Protection. By HUBERT MARTIN. 4th edition. Pp. viii + 359. London: Edward Arnold (Publishers) Ltd. 1959. 65*s*.

The term crop protection (now generally preferred to plant protection) has gradually come into established use as a convenient label for uniting certain aspects of mycology, plant pathology and entomology with weed control. But, as still occurs with the older names, it is being applied in a rather loose way and means different things to different people. If regarded as connoting the struggle to produce high-yielding crops as free as possible from pests, diseases and weeds it covers good husbandry as a basic essential, supplemented by the breeding of resistant varieties, biological control, the judicious use of insecticides, fungicides and herbicides, and other subsidiaries such as legislation and health certification schemes. Dr Martin deals briefly with some of these subjects but from the time he prepared the first edition of his book in 1928 he has never been in any doubt about his real aim, which has been to promote the co-operation between biologist and chemist so essential in crop protection, and to provide both with a survey of the whole field of chemical control.

In achieving that aim he has, of course, gone far beyond an outline of the scientific principles involved. Whether he has succeeded in providing the chemist 'with a means of approach to the biological side' is for the chemist to say, but generations of biologists are grateful to him for putting order into mental chaos by his clear exposition of the classification, characteristics, and mode of action of the ever-increasing number of groups of synthetic compounds being used in what, alas, is already being called the age of chemical farming. And the past thirty years has demonstrated beyond doubt that it is his book which has been constantly consulted by chemists and biologists alike as the essential guide to crop protection chemicals and the literature about them.

The new edition follows the familiar pattern of the earlier ones, and though the range of chemicals available has been greatly extended and the flow of literature unabated since the third edition appeared in 1940, the author has managed by skilful revision to compress his survey into approximately the same space, and to bring the information as nearly up-to-date as is possible with such an ever-changing subject.

W. C. MOORE

British Parasitic Fungi. By W. C. MOORE. Pp. 430. Cambridge University Press. 1959. 45*s*.

This is a book without a superfluous word. Part I, of 45 pages, catalogues alphabetically over 400 host plants (including trees) grown in Britain and gives all the fungus parasites recorded for each host. Part II (380 pages) lists all the parasites alphabetically, noting for each the original publication, the references in Saccardo's *Sylloge Fungorum*, the principal British references, and details of synonymy and imperfect stages. The pathogenic status of each fungus is defined and the diseases for which it is responsible are stated. Major diseases receive more extended treatment, with sections on history, epidemiology, spray treatment and resistant varieties. Such articles, for example, as those on *Phytophthora infestans*, *Venturia inaequalis* and *Botrytis cinerea* are masterpieces of succinct yet comprehensive presentation.

Not only the planning of the contents but also the typography, paper and binding ensure that the consultation of this book of reference can be made without either mental or optical strain. It is rare today to find a new book that, placed with its spine erect, will hold this position unsupported by any other volume. The squat shape of *British Parasitic Fungi* is indeed appropriate to this future bible of systematic plant pathologists.

R. W. MARSH

Blakeslee: the genus Datura. By A. G. AVERY, S. SATINA and J. RIETSEMA. 1959. Pp. xli + 289, with 34 tables and 68 illustrations in the text. New York: Ronald Press. Price $8.75.

Albert F. Blakeslee (1874–1954) spent much of his long life working on *Datura*. He gathered around him an able group of investigators, among whom the three authors of this volume were numbered. The book is essentially a critical synthesis of all the *Datura* investigations, with the additional function of honouring their initiator. It begins with a foreword and a biographical sketch of Blakeslee by E. W. Sinnott. The three authors take individual responsibility for the fifteen chapters, assisted in several instances by four collaborators, M.-H. Sachet, E. G. Leete, J. M. Cummings and B. Blondel; despite the diversity of authorship, a satisfactory uniformity of style and presentation is achieved. There are two major bibliographies—one (arranged historically) of papers by Blakeslee and his associates, the other general; and an adequate index.

Work on *Datura* began in 1915, soon after the early triumphs of the Morgan school. Fortified by the brilliant application of the acetocarmine technique by Belling, trisomics, interchanges, polyploids and the first recorded plant haploid were soon discovered and *Datura* quickly became a leading organism in the explosive evolution that cytogenetics was then undergoing; the trisomic studies and the analysis of interchange in relation to taxonomy are still the most elegant researches of their kind available. But, gradually—for reasons mentioned below—*Datura* and formal cytogenetics tended to part company though, to be sure, the work retained its cytogenetic bias until the end. The new trend was physiological and a splendid series of investigations of pollen behaviour, tissue genesis (by means of mixoploid chimeras), seed and embryo development and the nature of interspecific mating barriers followed. The trend is well illustrated by the fact that virtually all the papers listed at the beginning of the historical bibliography are cytogenetical in content, whereas over 50% of those at the end of the same bibliography are physiological.

As an experimental organism, *Datura* is excellent for some purposes, not so good for others. It is quick and easy to grow and is comparatively free of disease; it has large flowers and many seeds per capsule; the seeds retain viability well and plants can be easily grafted; not least important, it has workable metaphase chromosomes. *Drosophila* and maize were important objects of cytogenetical research long before the technical potentialities of their chromosomes (salivary or pachytene) were realized. If *Datura* had had a pachytene as workable as that of maize, the history of cytogenetics might have been rather different. I think it reasonable to suggest that the physiological trend found in the later years of *Datura* research was determined not only by its very great intrinsic interest but also by the realization that the major cytogenetic questions of the 1930's and '40's were better answered with the aid of other organisms, conspicuously maize and *Drosophila*; nowadays, these in turn have been superseded for many purposes.

For all the splendid contributions made by the *Datura* work, it is well to appreciate that there are certain important areas on which it had little or no impact: on biochemical genetics, chromosome mechanics and population genetics, for example. But within the chosen fields of study, the *Datura* contribution is second to none; we may be grateful to the authors who have presented a lucid summary of the work, a fitting memorial to a very distinguished investigator; and to the publishers for an excellent job of production.

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